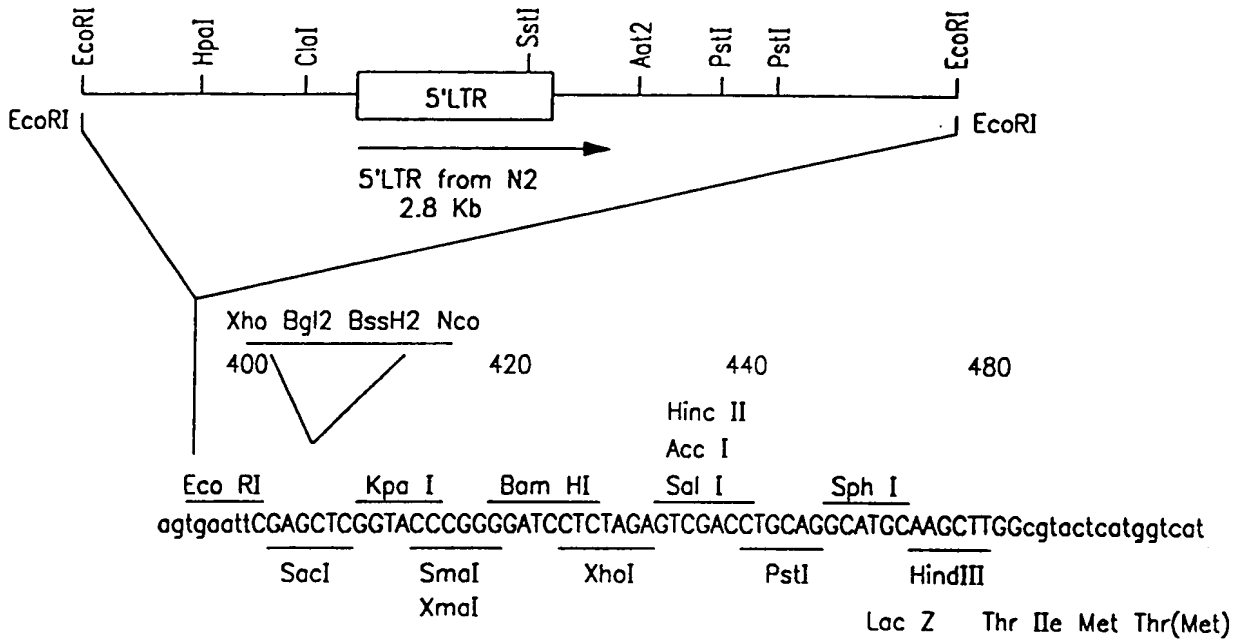

EcoRI
HpaI
ClaI
SstI
Aat2
PstI
PstI
EcoRI
5'LTR
EcoRI
EcoRI
5'LTR from N2
2.8 Kb
Xho Bgl2 BssH2 Nco
400
420
440
480
Hinc II
Acc I
Eco RI
Kpa I
Bam HI
Sal I
Sph I
agtgaattCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGcgtactcatggtcat
SacI
SmaI
XmaI
XhoI
PstI
HindIII
Lac Z
Thr Ile Met Thr(Met)

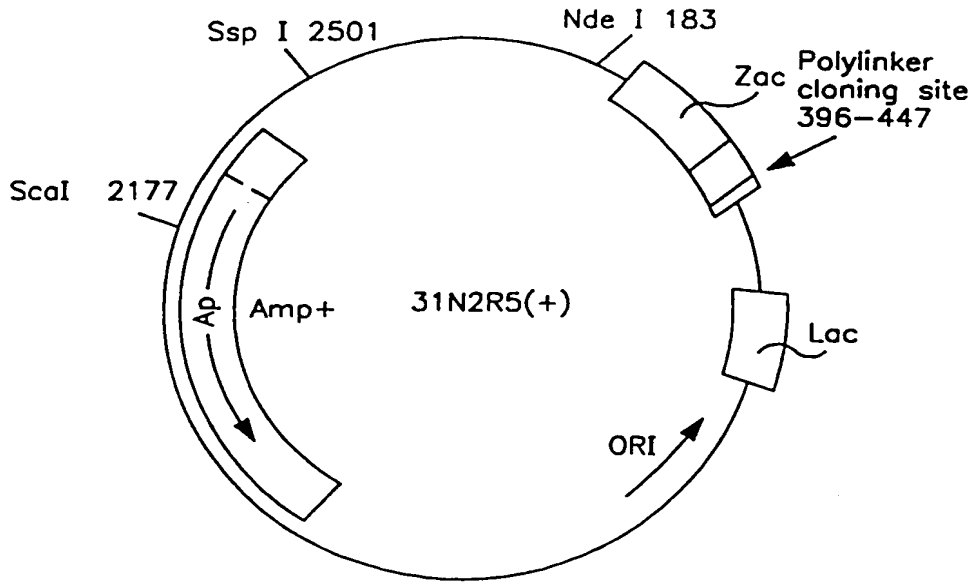

Ssp I 2501
Nde I 183
Zac
Polylinker
cloning site
396–447
ScaI 2177
Ap
Amp+
31N2R5(+)
Lac
ORI

FIG. 1

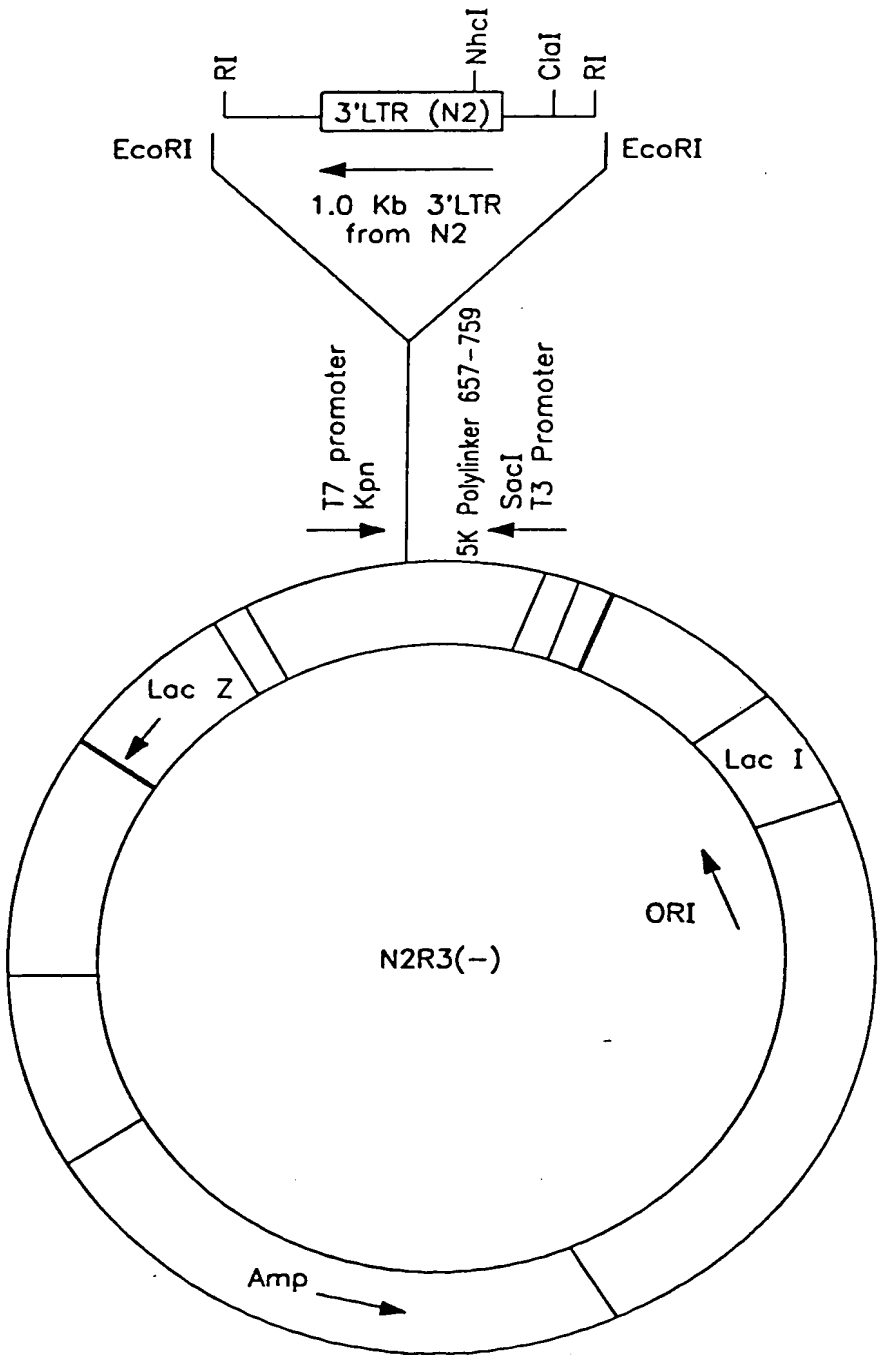
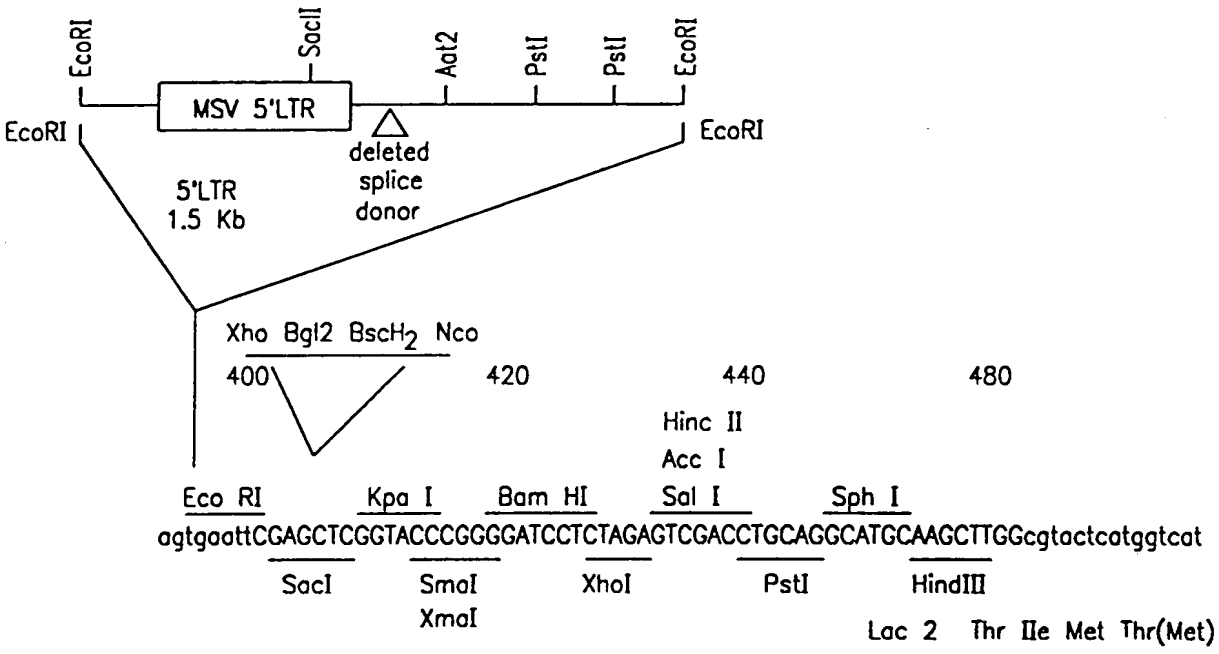

RI
NhcI
ClaI
RI
3'LTR (N2)
EcoRI
EcoRI
1.0 Kb 3'LTR
from N2
T7 promoter
Kpn
5K Polylinker 657-759
SacI
T3 Promoter
Lac Z
Lac I
ORI
N2R3(-)
Amp

FIG. 2


EcoRI
SacII
Aat2
PstI
PstI
EcoRI
MSV 5'LTR
EcoRI
EcoRI
deleted
splice
donor
5'LTR
1.5 Kb
Xho Bgl2 BscH2 Nco
400
420
440
480
Hinc II
Acc I
Eco RI
Kpa I
Bam HI
Sal I
Sph I
agtgaattCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGcgtactcatggtcat
SacI
SmaI
XmaI
XhoI
PstI
HindIII
Lac 2 Thr Ile Met Thr(Met)

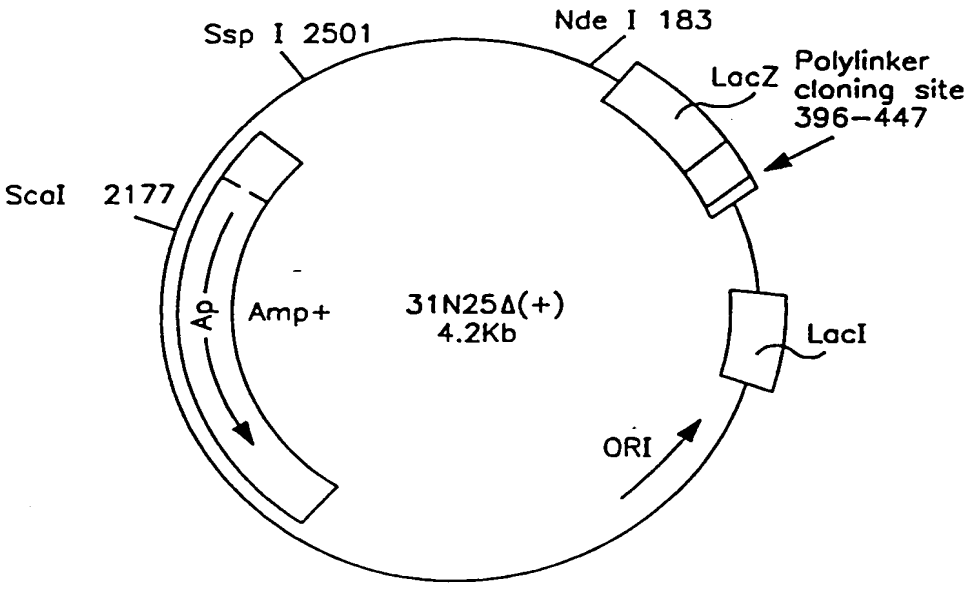

Ssp I 2501
Nde I 183
LacZ
Polylinker
cloning site
396–447
ScaI 2177
Ap
Amp+
31N25Δ(+)
4.2Kb
LacI
ORI

FIG. 3

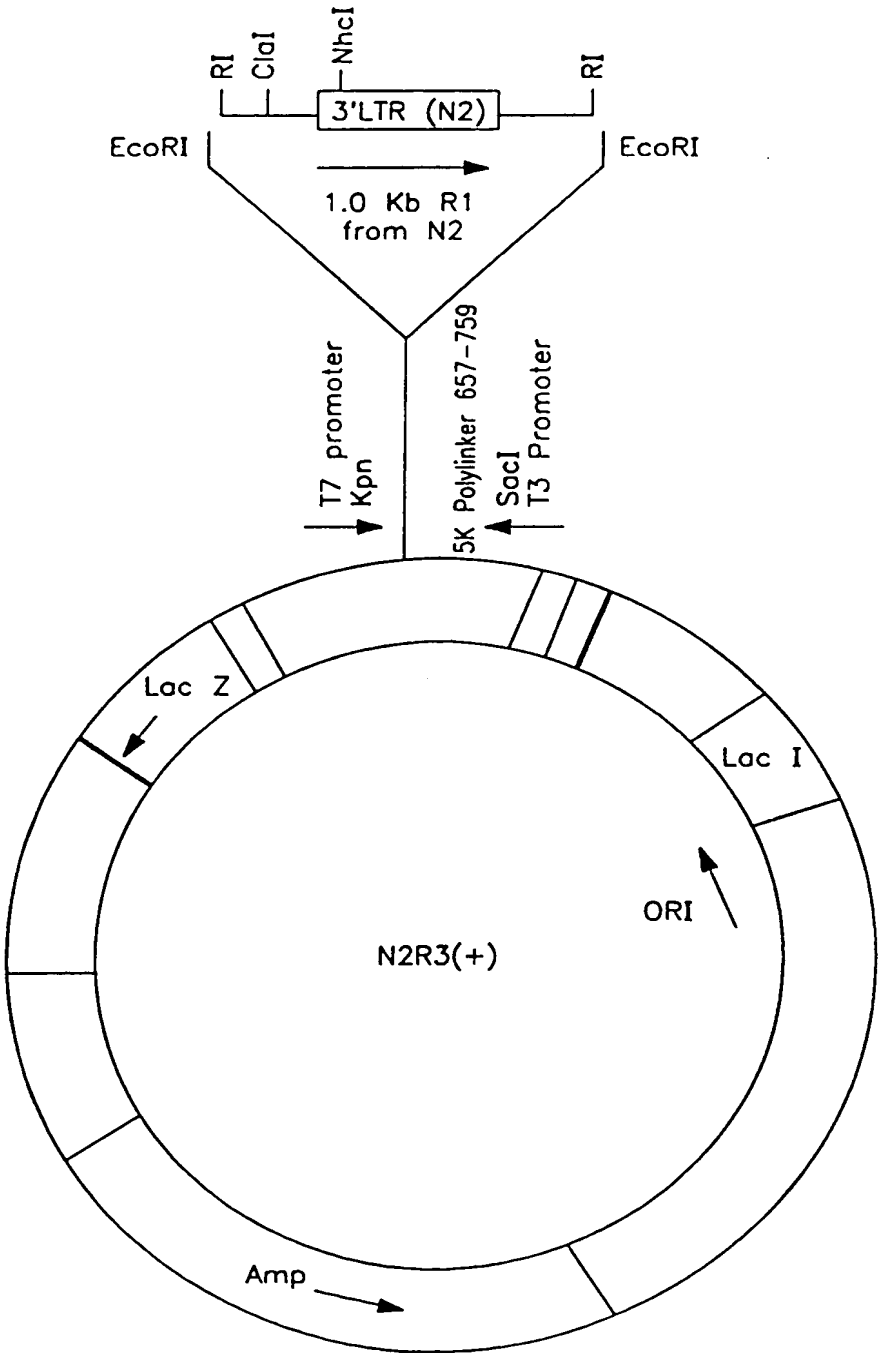

RI
ClaI
NhcI
RI
3'LTR (N2)
EcoRI
EcoRI
1.0 Kb R1
from N2
T7 promoter
Kpn
5K Polylinker 657-759
SacI
T3 Promoter
Lac Z
Lac I
ORI
N2R3(+)
Amp

FIG. 4

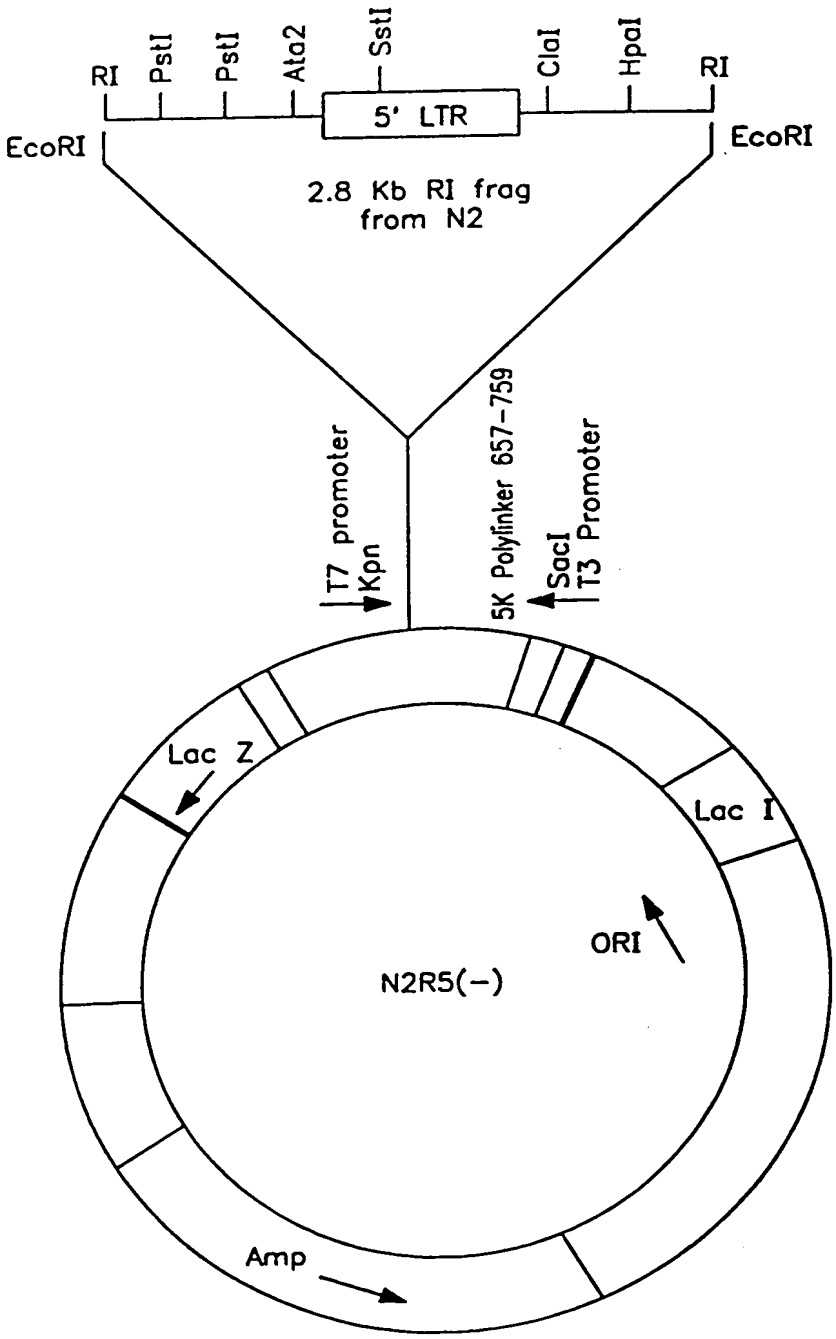
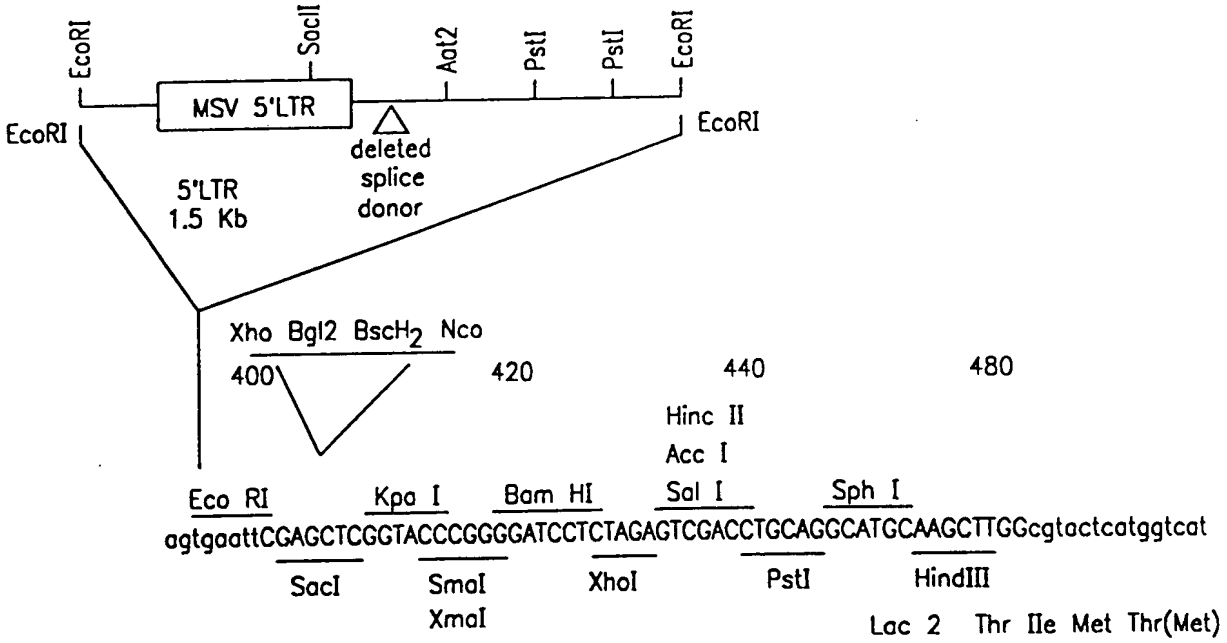

RI
PstI
PstI
Ata2
SstI
ClaI
HpaI
RI
5' LTR
EcoRI
EcoRI
2.8 Kb RI frag
from N2
T7 promoter
Kpn
5K Polylinker 657-759
SacI
T3 Promoter
Lac Z
Lac I
ORI
N2R5(-)
Amp

FIG. 5


EcoRI
SacII
Aat2
PstI
PstI
EcoRI
MSV 5'LTR
EcoRI
EcoRI
deleted
splice
donor
5'LTR
1.5 Kb
Xho Bgl2 BscH2 Nco
400
420
440
480
Hinc II
Acc I
Eco RI
Kpn I
Bam HI
Sal I
Sph I
agtgaattCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGcgtactcatggtcat
SacI
SmaI
XmaI
XhoI
PstI
HindIII
Lac 2 Thr Ile Met Thr(Met)

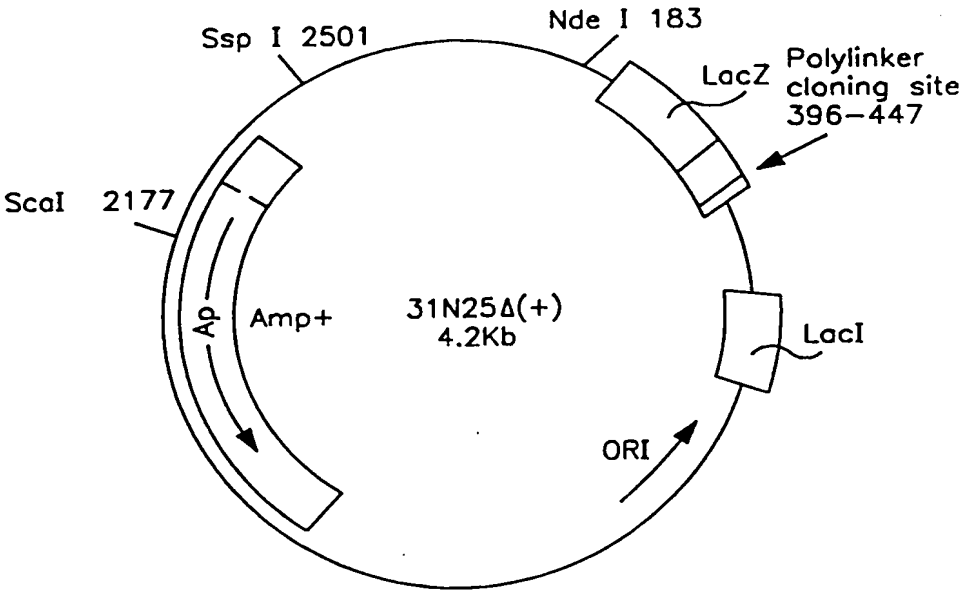

Ssp I 2501
Nde I 183
LacZ
Polylinker
cloning site
396–447
ScaI 2177
Ap
Amp+
31N25Δ(+)
4.2Kb
LacI
ORI

FIG. 6

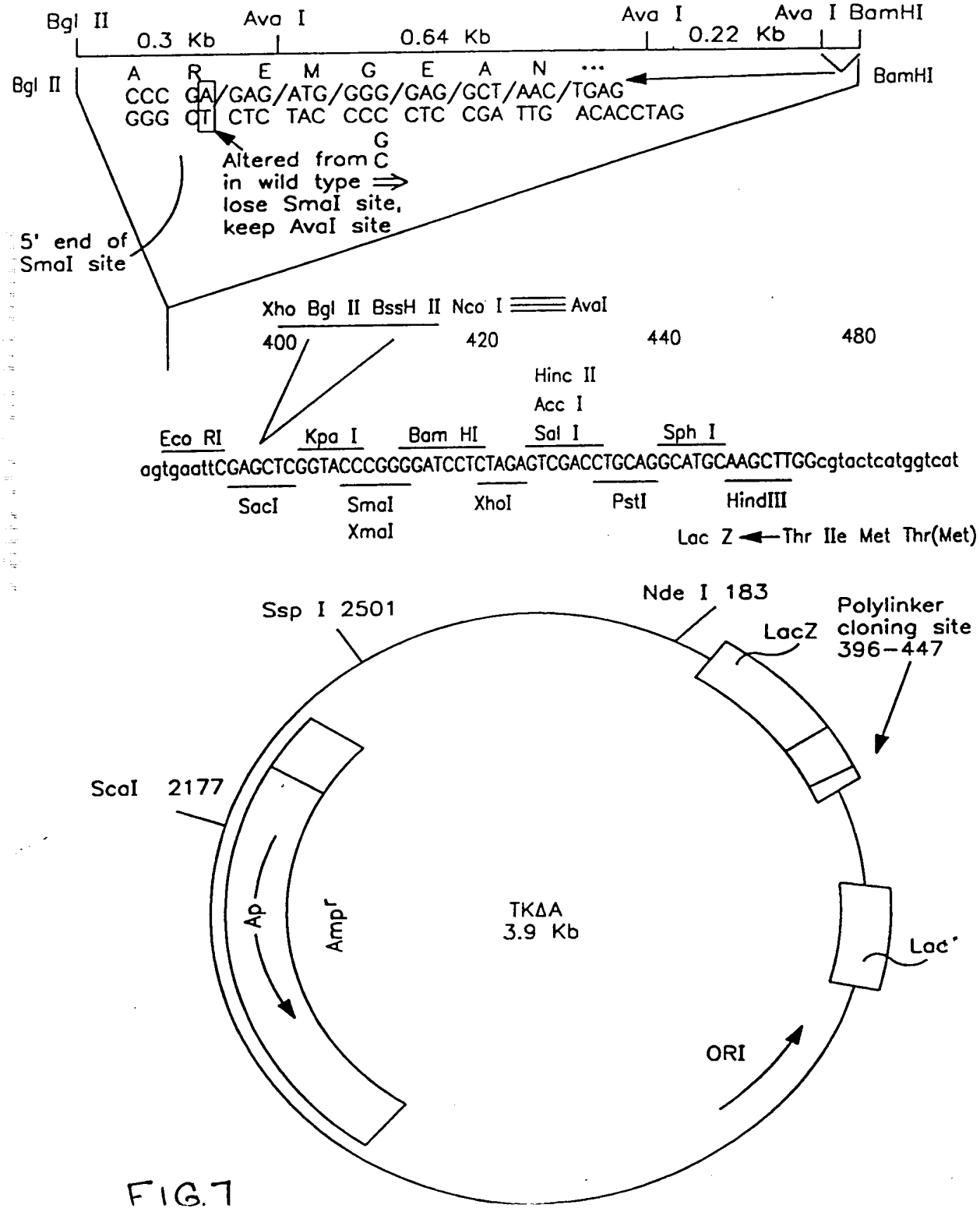

Bgl II
Ava I
Ava I
Ava I BamHI
0.3 Kb
0.64 Kb
0.22 Kb
A R E M G E A N ...
Bgl II
CCC GA/GAG/ATG/GGG/GAG/GCT/AAC/TGAG
GGG CT CTC TAC CCC CTC CGA TTG ACACCTAG
BamHI
G
Altered from C
in wild type ⇒
lose SmaI site,
keep AvaI site
5' end of
SmaI site
Xho Bgl II BssH II Nco I AvaI
400
420
440
480
Hinc II
Acc I
Eco RI
Kpa I
Bam HI
Sal I
Sph I
agtgaattCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGcgtactcatggtcat
SacI
SmaI
XmaI
XhoI
PstI
HindIII
Lac Z ← Thr Ile Met Thr(Met)
Ssp I 2501
Nde I 183
LacZ
Polylinker
cloning site
396–447
ScaI 2177
Ap
Ampr
TKΔA
3.9 Kb
Lac'
ORI

FIG.7

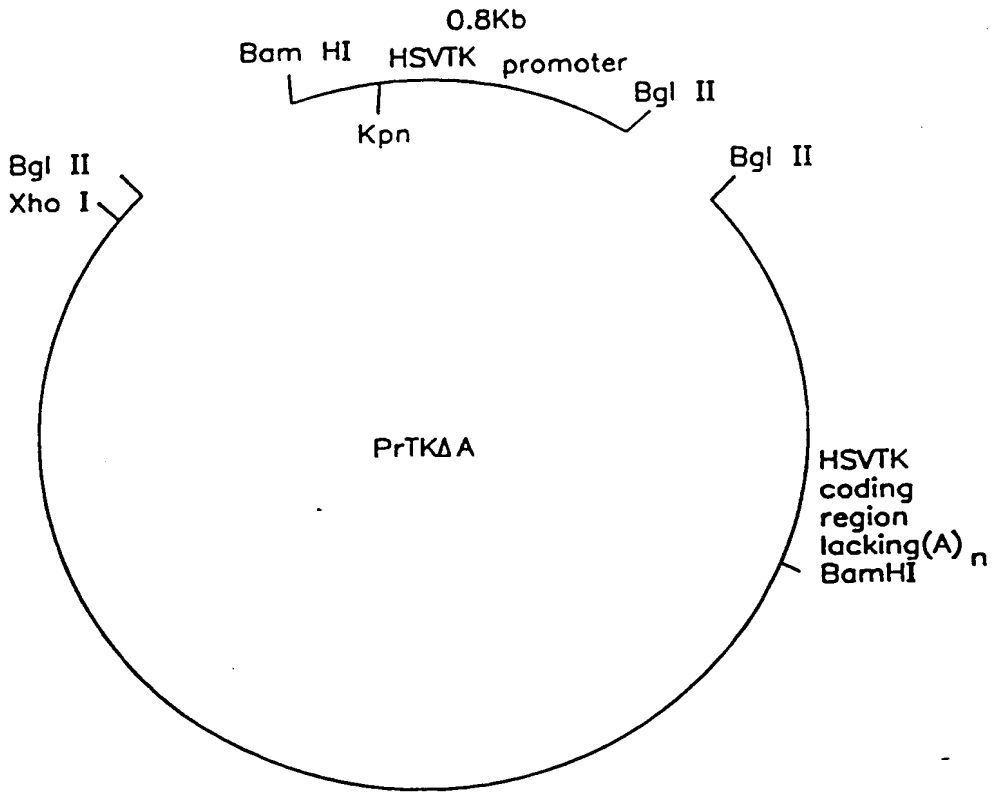

0.8Kb
Bam HI
HSVTK promoter
Bgl II
Kpn
Bgl II
Xho I
Bgl II
PrTKΔA
HSVTK
coding
region
lacking(A) n
BamHI

FIG. 8

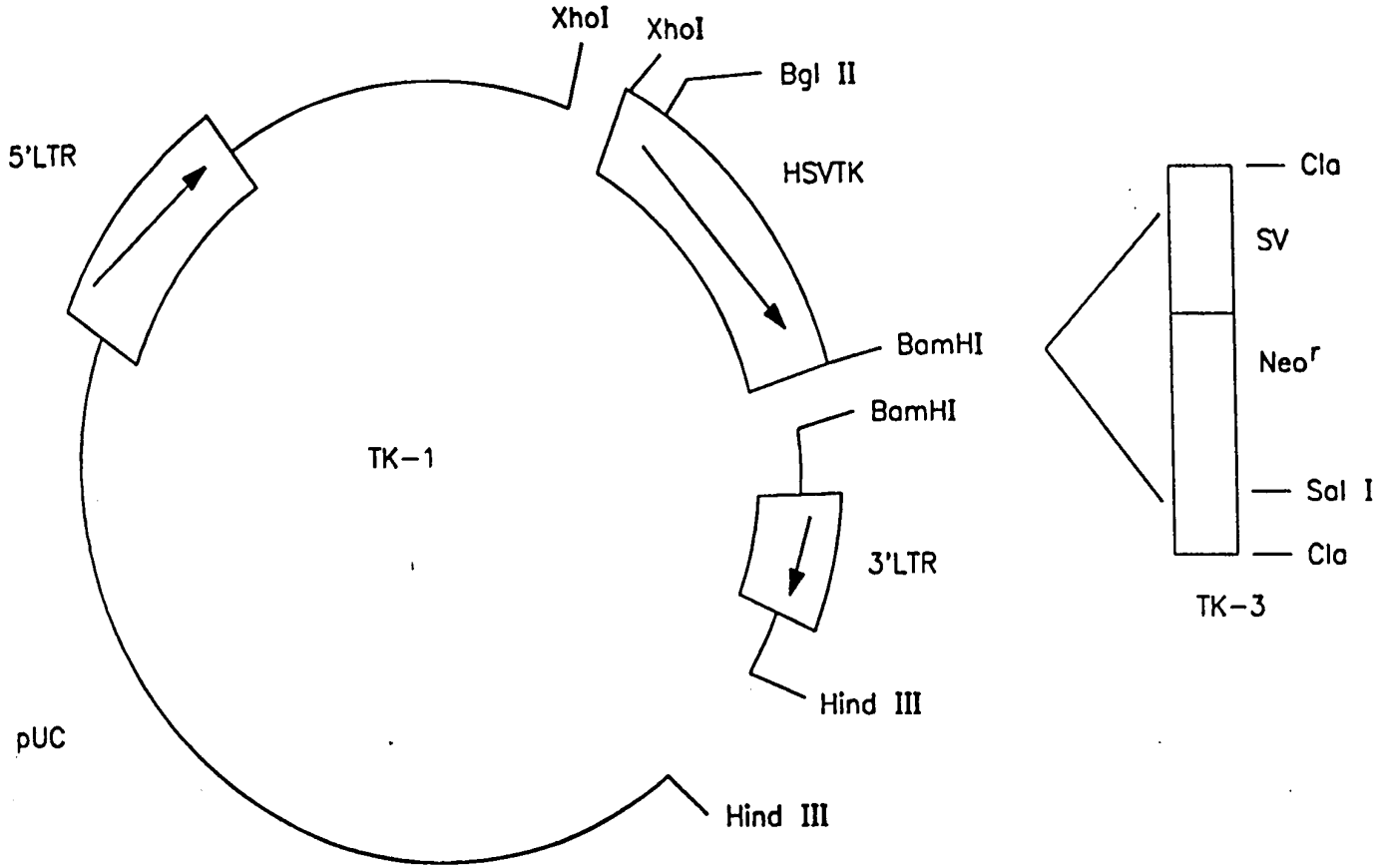

XhoI
XhoI
Bgl II
HSVTK
5'LTR
BamHI
BamHI
TK-1
3'LTR
Hind III
pUC
Hind III
Cla
SV
Neo^r
Sal I
Cla
TK-3

FIG. 9

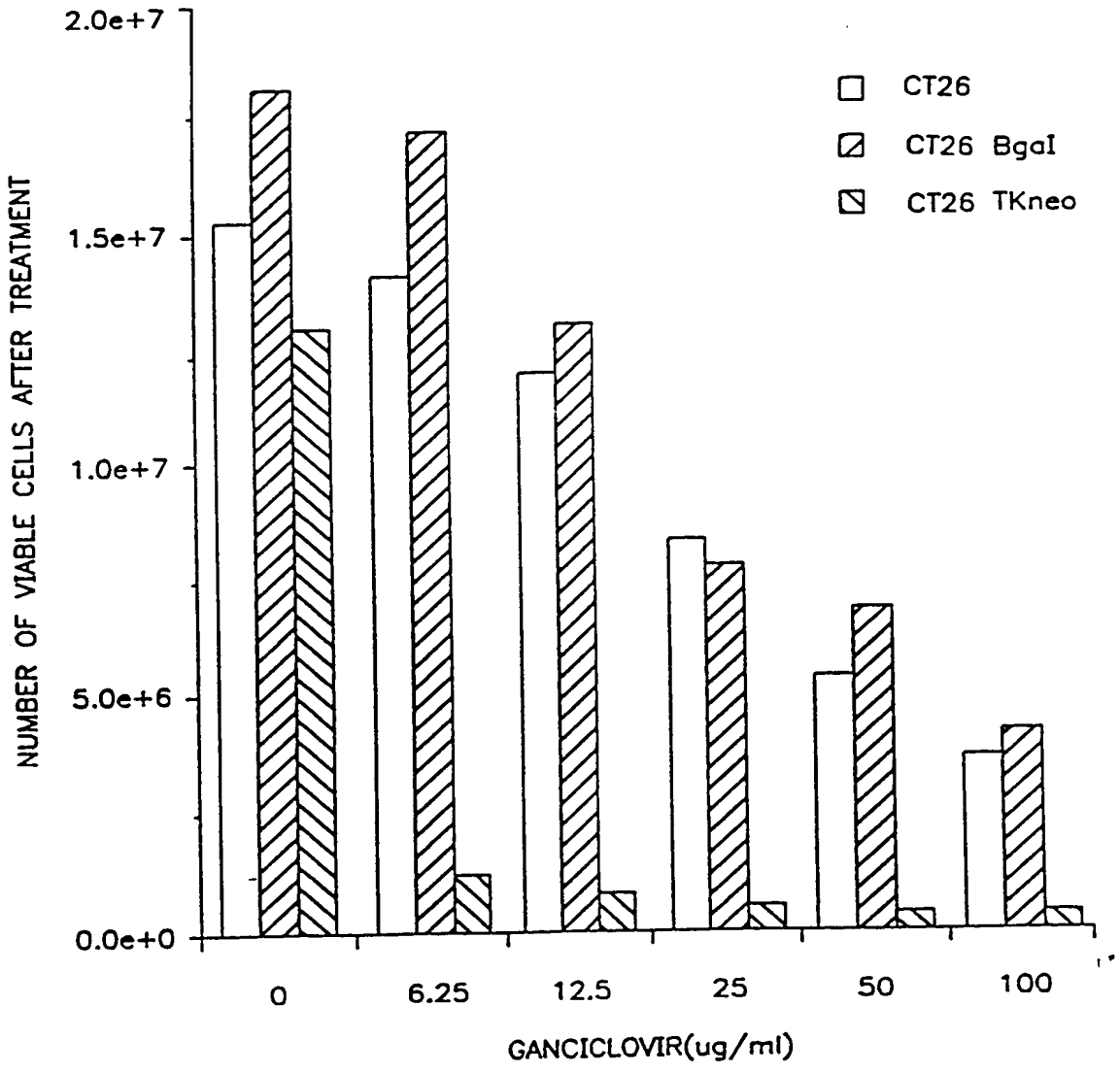

2.0e+7
1.5e+7
1.0e+7
5.0e+6
0.0e+0
NUMBER OF VIABLE CELLS AFTER TREATMENT
CT26
CT26 BgaI
CT26 TKneo
0
6.25
12.5
25
50
100
GANCICLOVIR(ug/ml)

FIG. 10

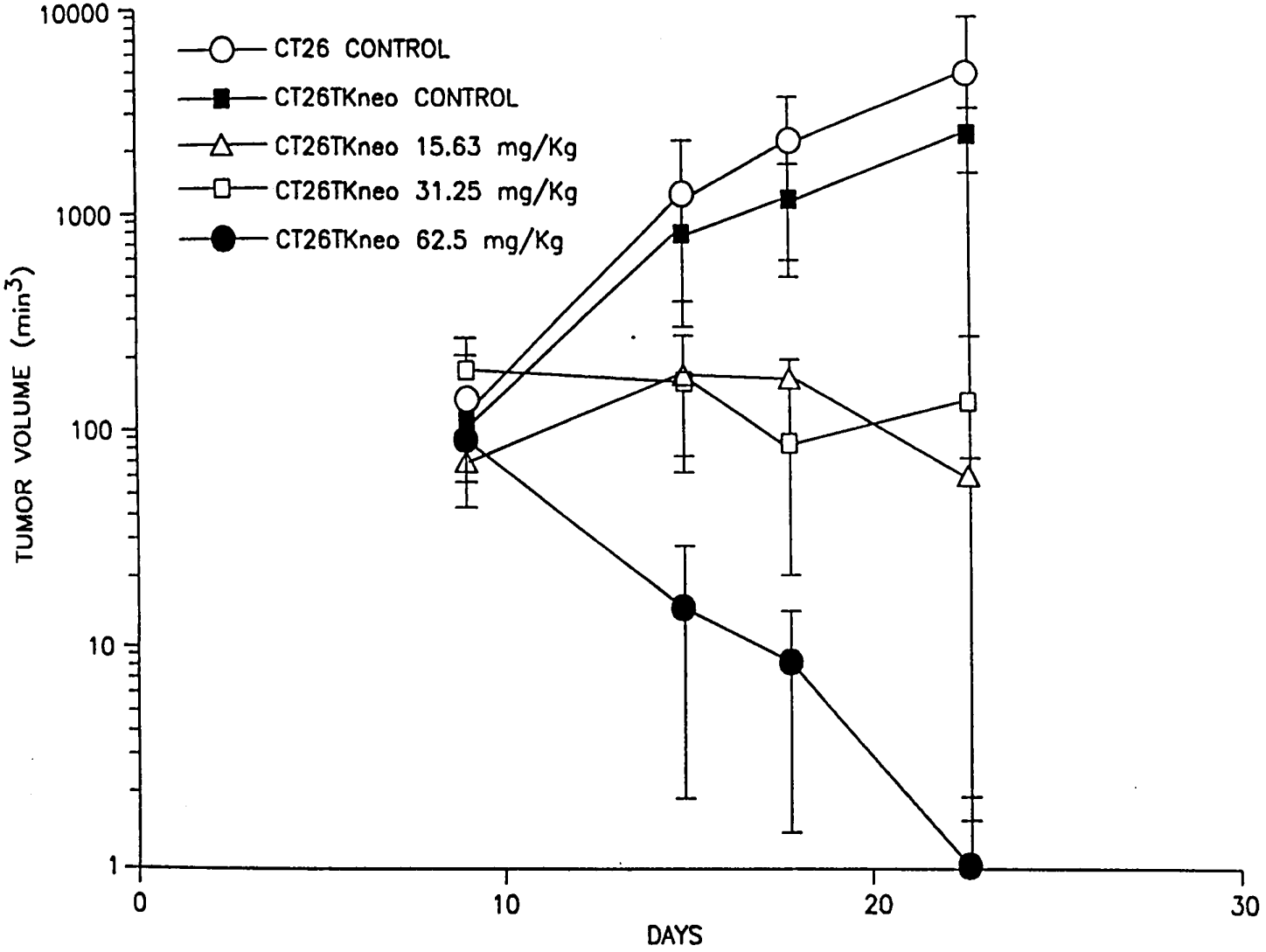

10000
1000
100
10
1
TUMOR VOLUME (min3)
CT26 CONTROL
CT26TKneo CONTROL
CT26TKneo 15.63 mg/Kg
CT26TKneo 31.25 mg/Kg
CT26TKneo 62.5 mg/Kg
0
10
20
30
DAYS

FIG. II

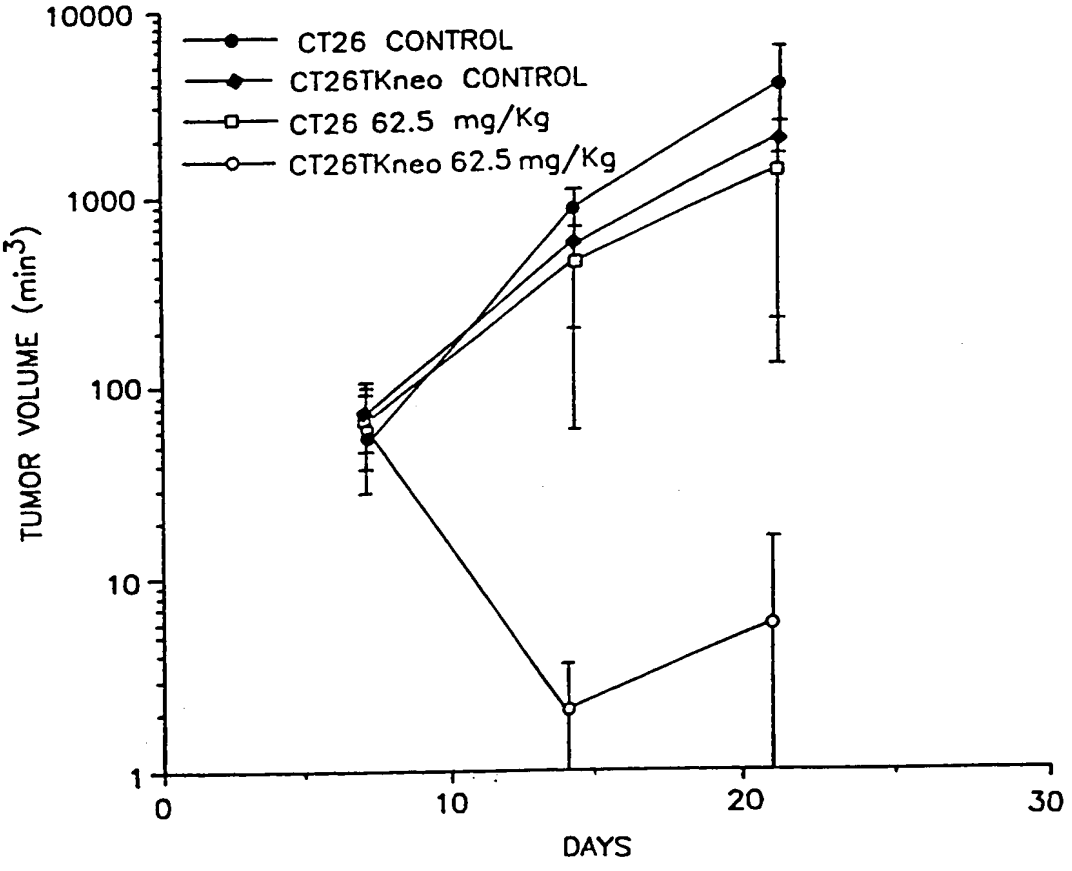
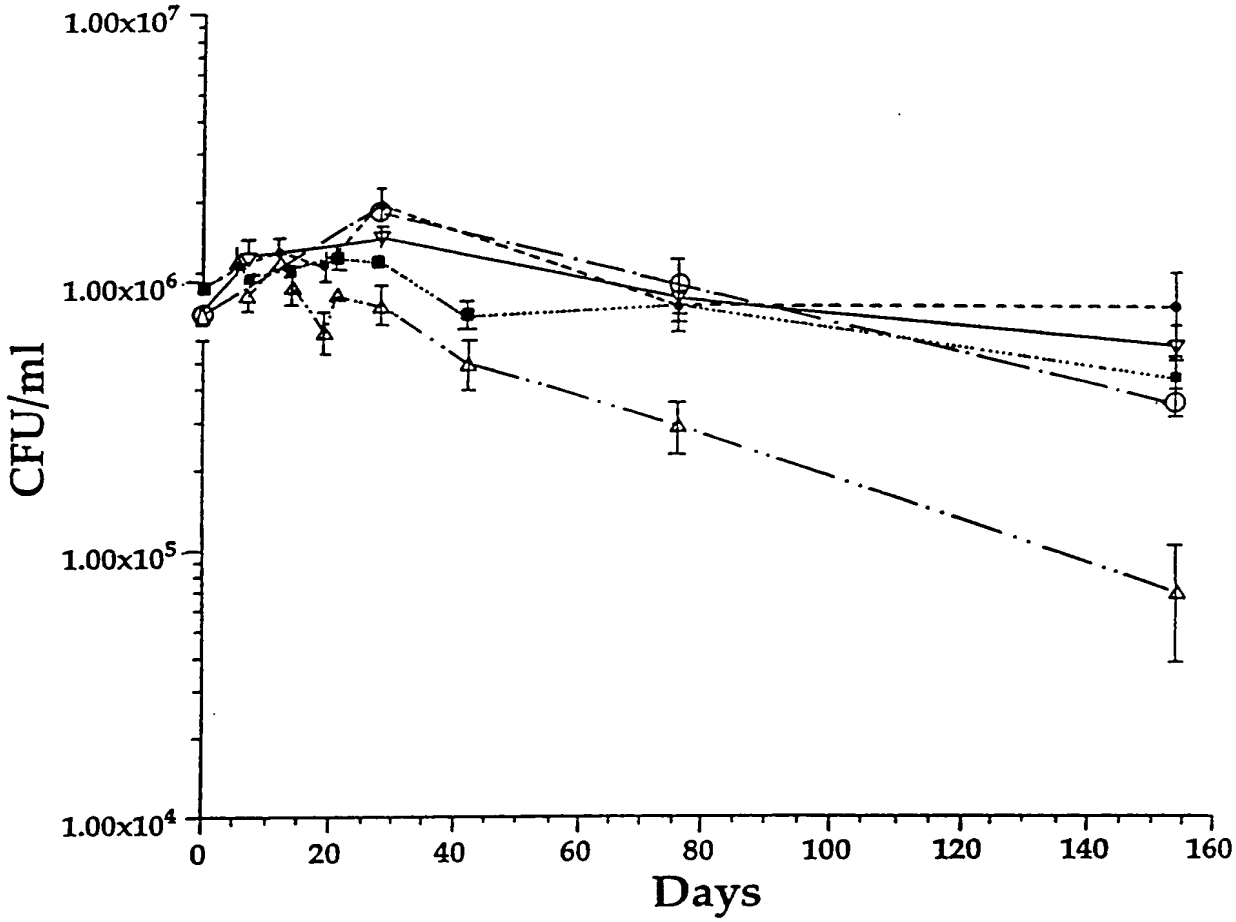

10000
1000
100
10
1
TUMOR VOLUME (min3)
CT26 CONTROL
CT26TKneo CONTROL
CT26 62.5 mg/Kg
CT26TKneo 62.5 mg/Kg
0
10
20
30
DAYS

fig 12

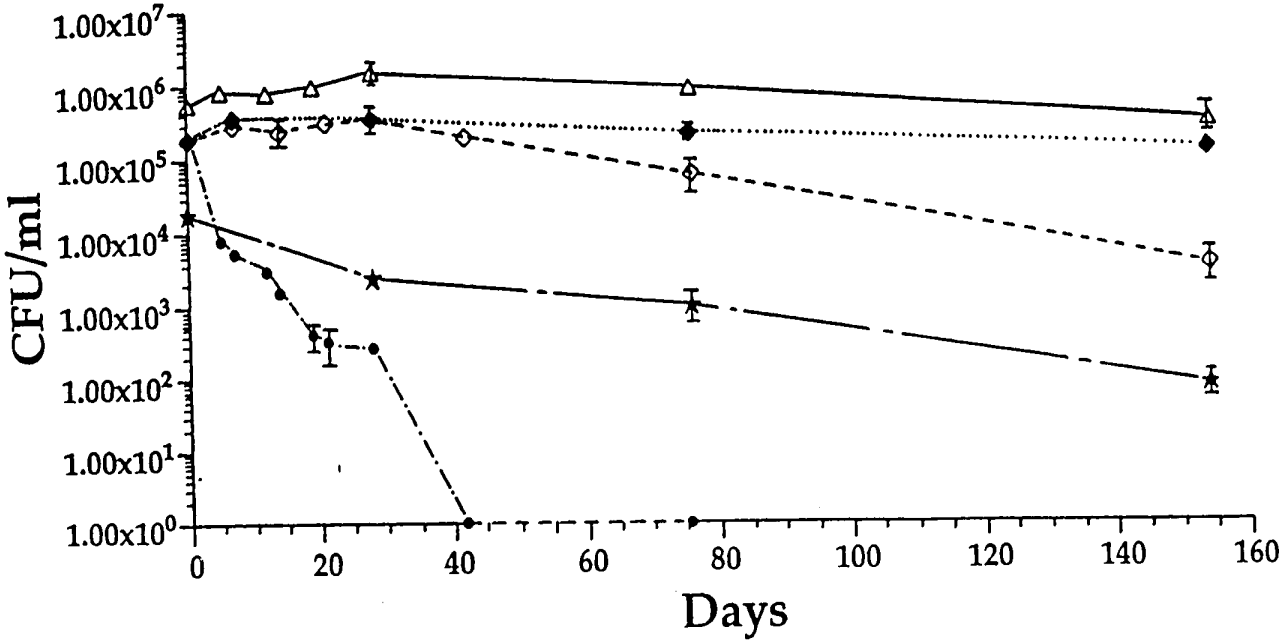


Formulation:

25mM Tris pH 7.2
60mM NaCl
1 mg/ml Arginine
5 mg/ml HSA
50 mg/ml Lactose

-80°C Liquid
-20°C Liquid
-20°C Lyoph
Refrig. Lyoph
R.T. Lyoph

FIG. 13

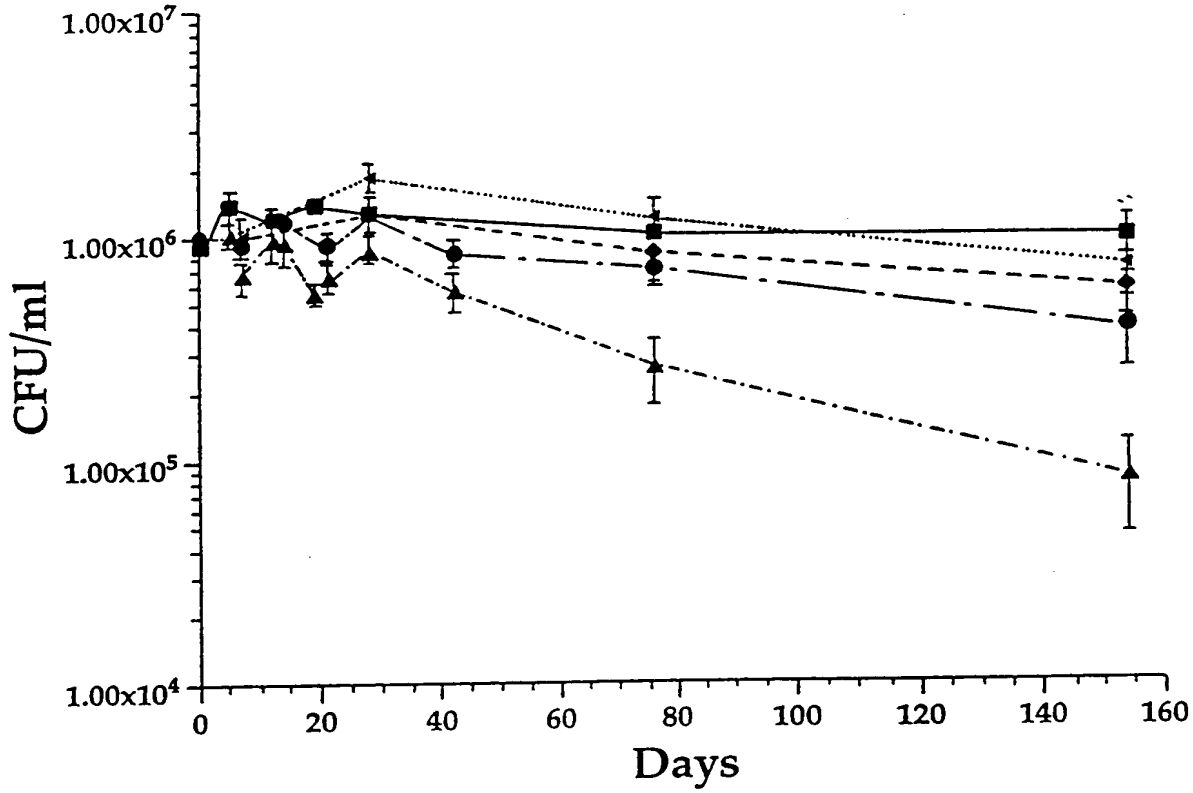


Formulation:

25mM Tris pH 7.2
25mM NaCl
1 mg/ml Arginine
5 mg/ml HSA
40 mg/ml Mannitol

-80°C Liquid
-20°C Liquid
-20°C Lyoph
Refrig. Lyoph
R.T. Lyoph

FIG. 14



Formulation:
25mM Tris pH 7.2
60mM NaCl
1mg/ml Arginine
5mg/ml HSA
50mg/ml Trehalose

-80°C Liquid
-20°C Liquid
-20°C Lyoph
Refrig. Lyoph
R.T. Lyoph

FIG. 15

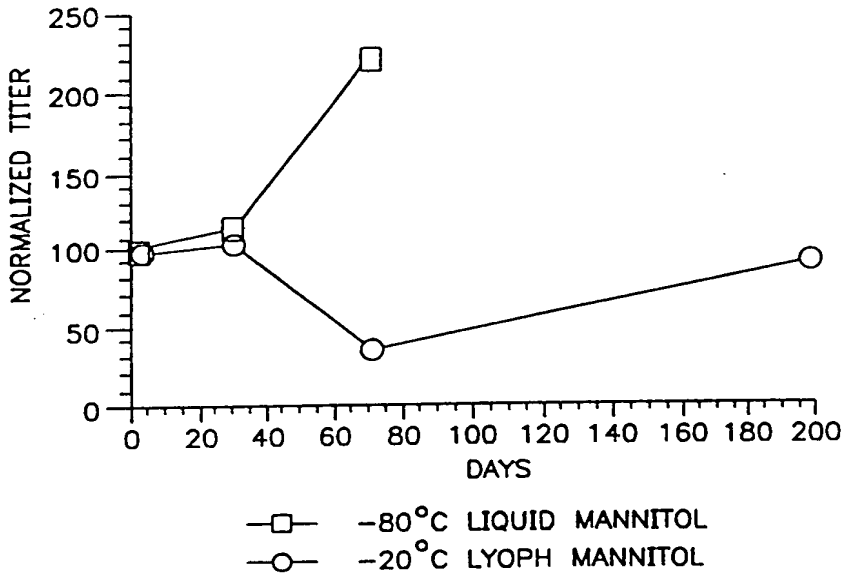


FIG. 16A

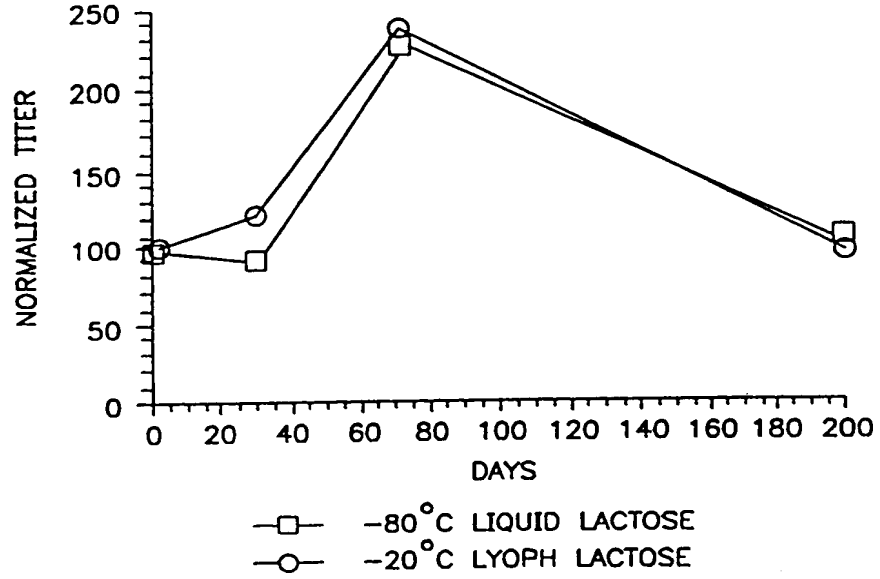


FIG. 16B

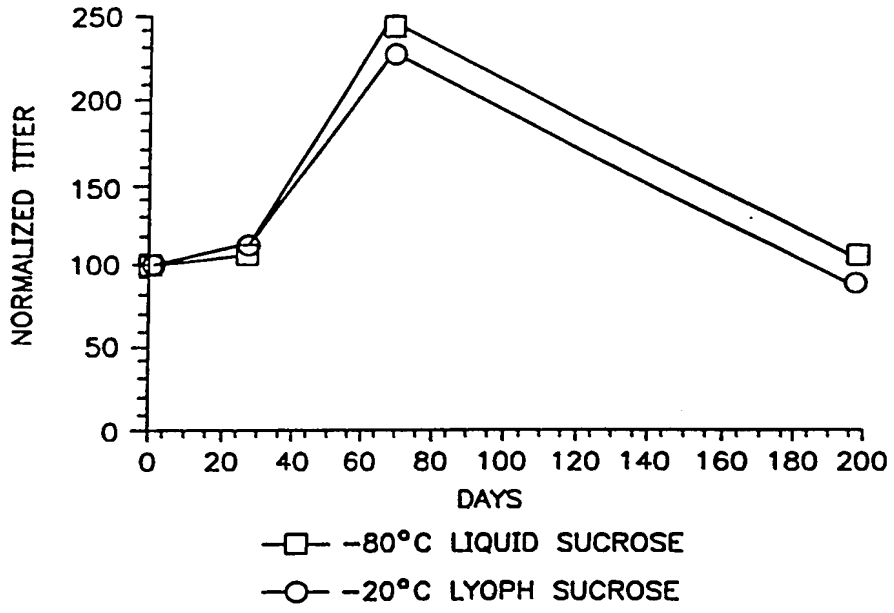


FIG. 16C

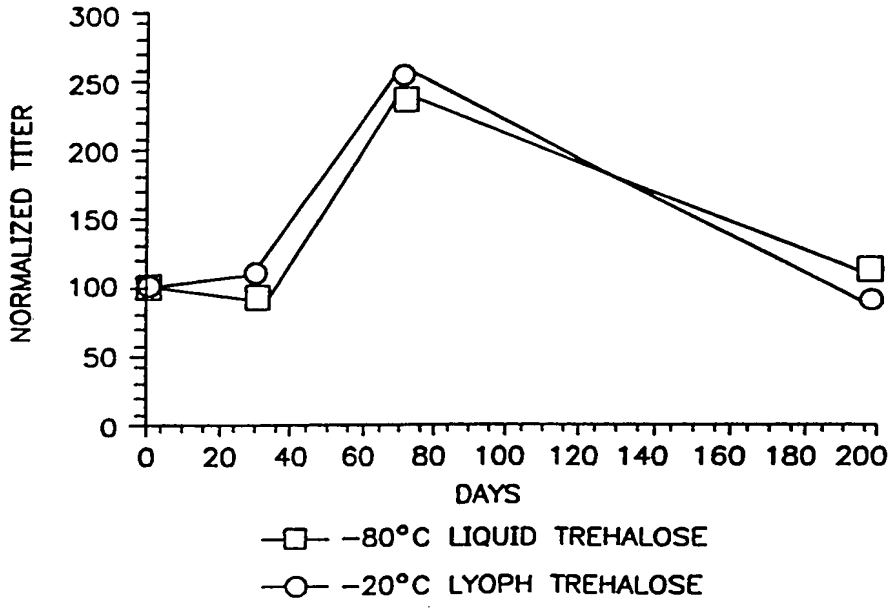


FIG. 16D

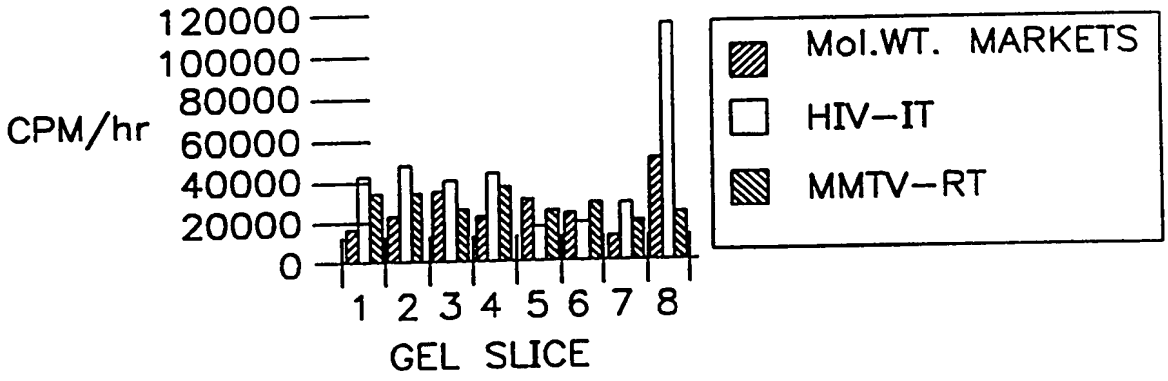

120000
100000
80000
60000
40000
20000
0
CPM/hr
1 2 3 4 5 6 7 8
GEL SLICE
Mol.WT. MARKETS
HIV-IT
MMTV-RT

FIG. 17

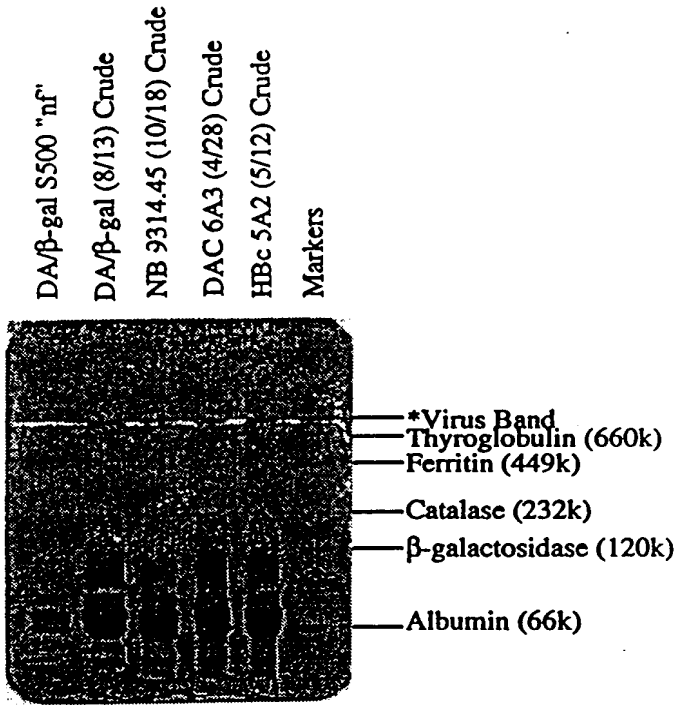

DA/β-gal S500 "nf"
DA/β-gal (8/13) Crude
NB 9314.45 (10/18) Crude
DAC 6A3 (4/28) Crude
HBc 5A2 (5/12) Crude
Markers
*Virus Band
Thyroglobulin (660k)
Ferritin (449k)
Catalase (232k)
β-galactosidase (120k)
Albumin (66k)

FIG. 18

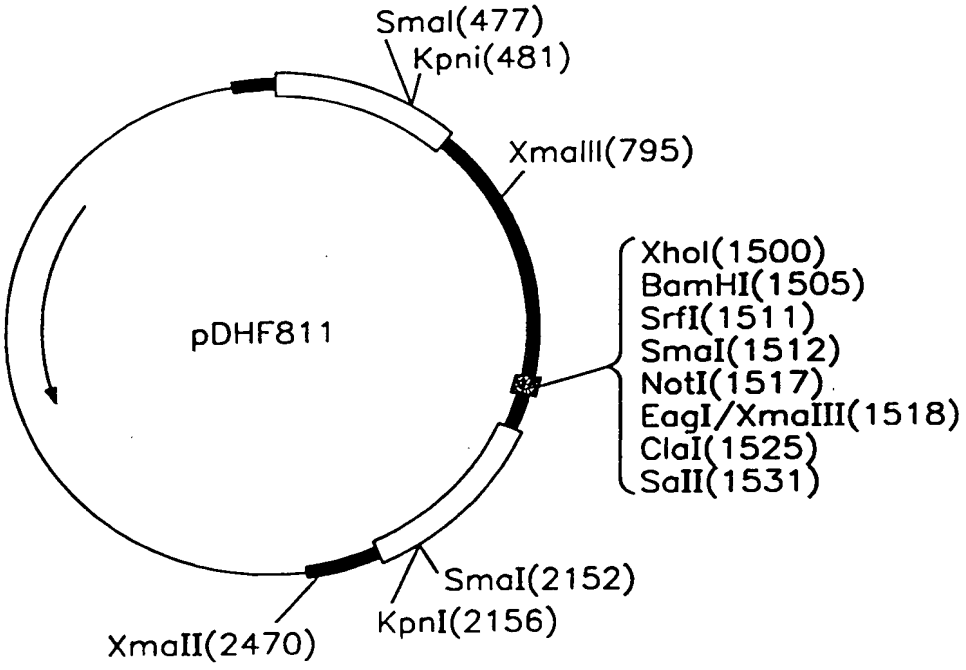

SmaI(477)
KpnI(481)
XmaIII(795)
XhoI(1500)
BamHI(1505)
SrfI(1511)
SmaI(1512)
NotI(1517)
EagI/XmaIII(1518)
ClaI(1525)
SalI(1531)
pDHF811
SmaI(2152)
KpnI(2156)
XmaII(2470)

FIG. 19

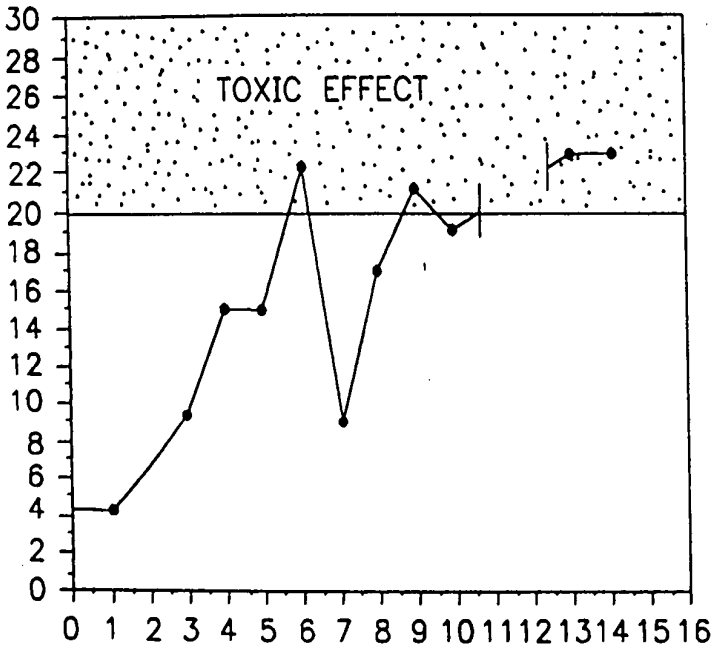


FIG. 20A

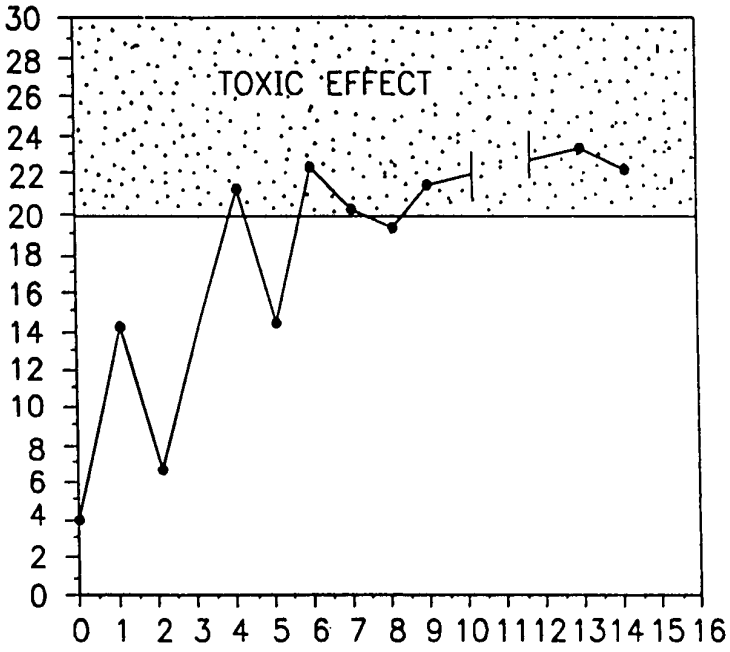


FIG. 20B

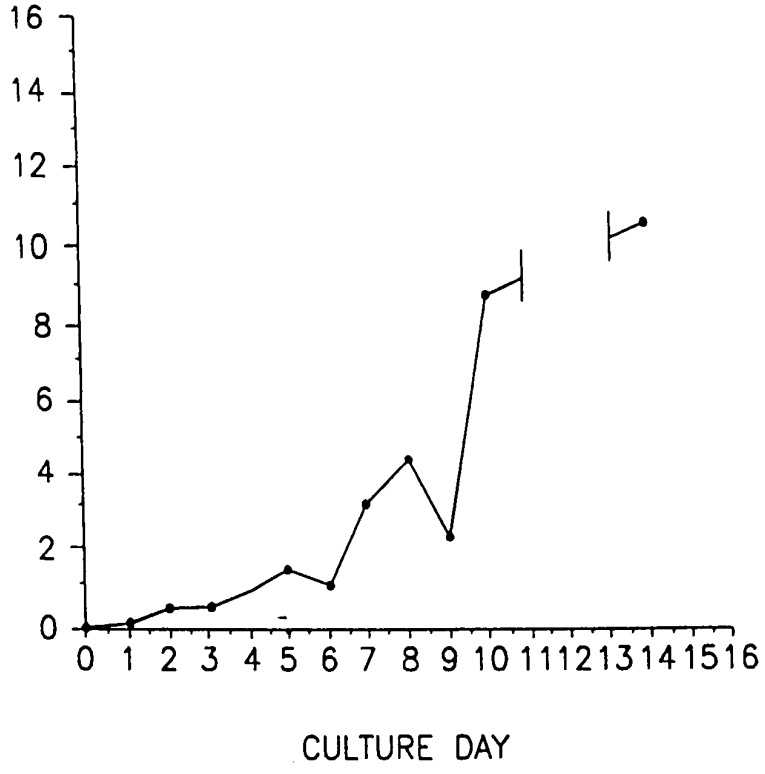


FIG. 20C

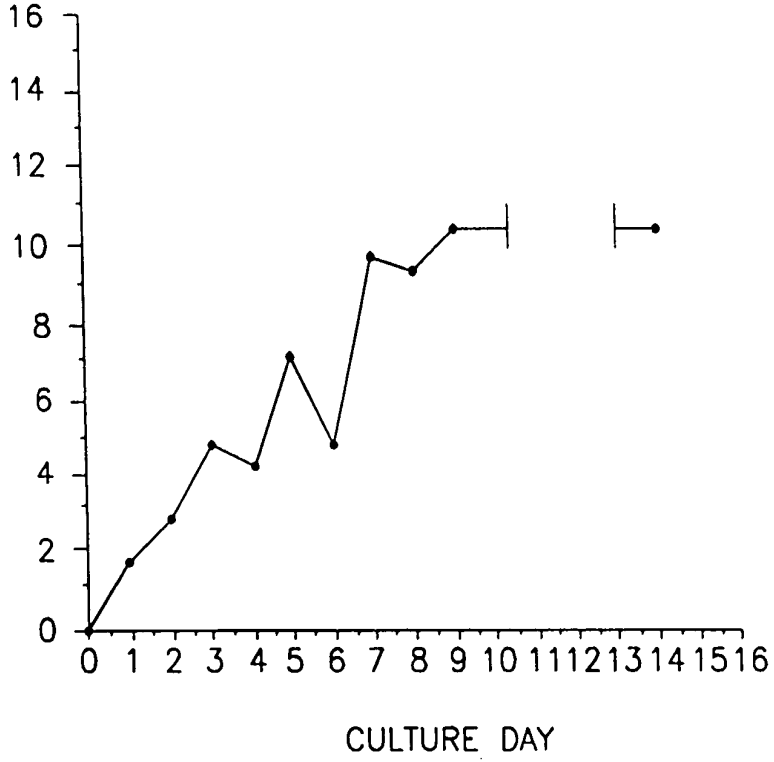


FIG. 20D

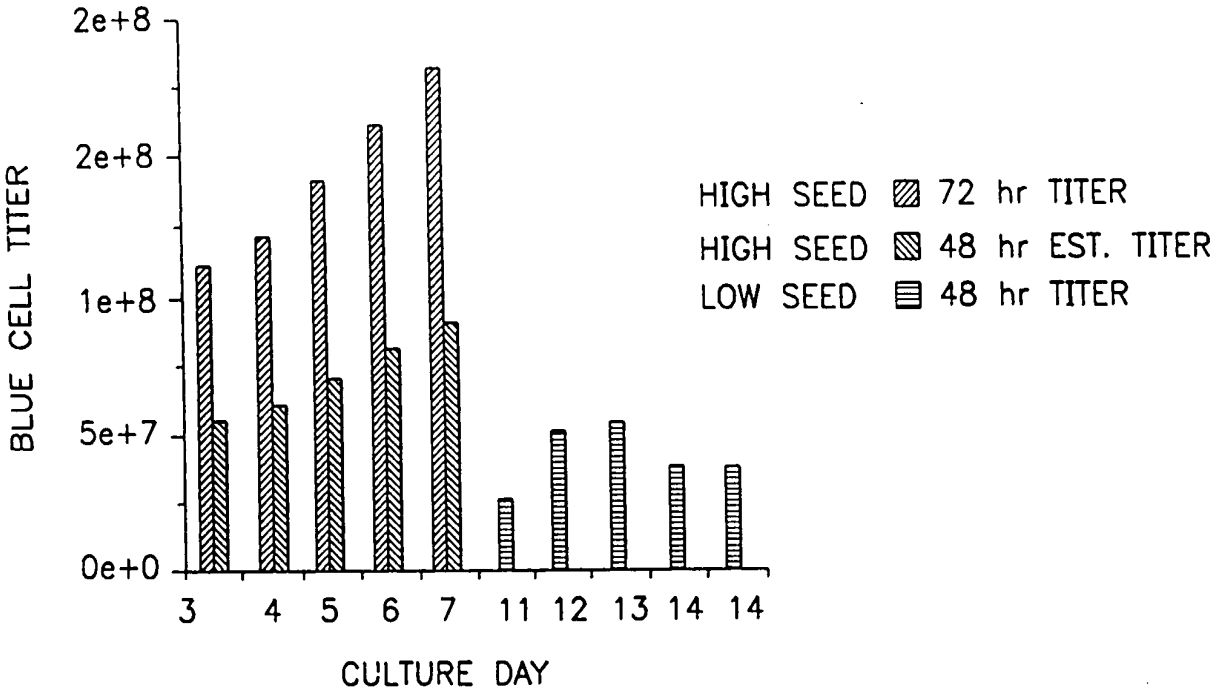
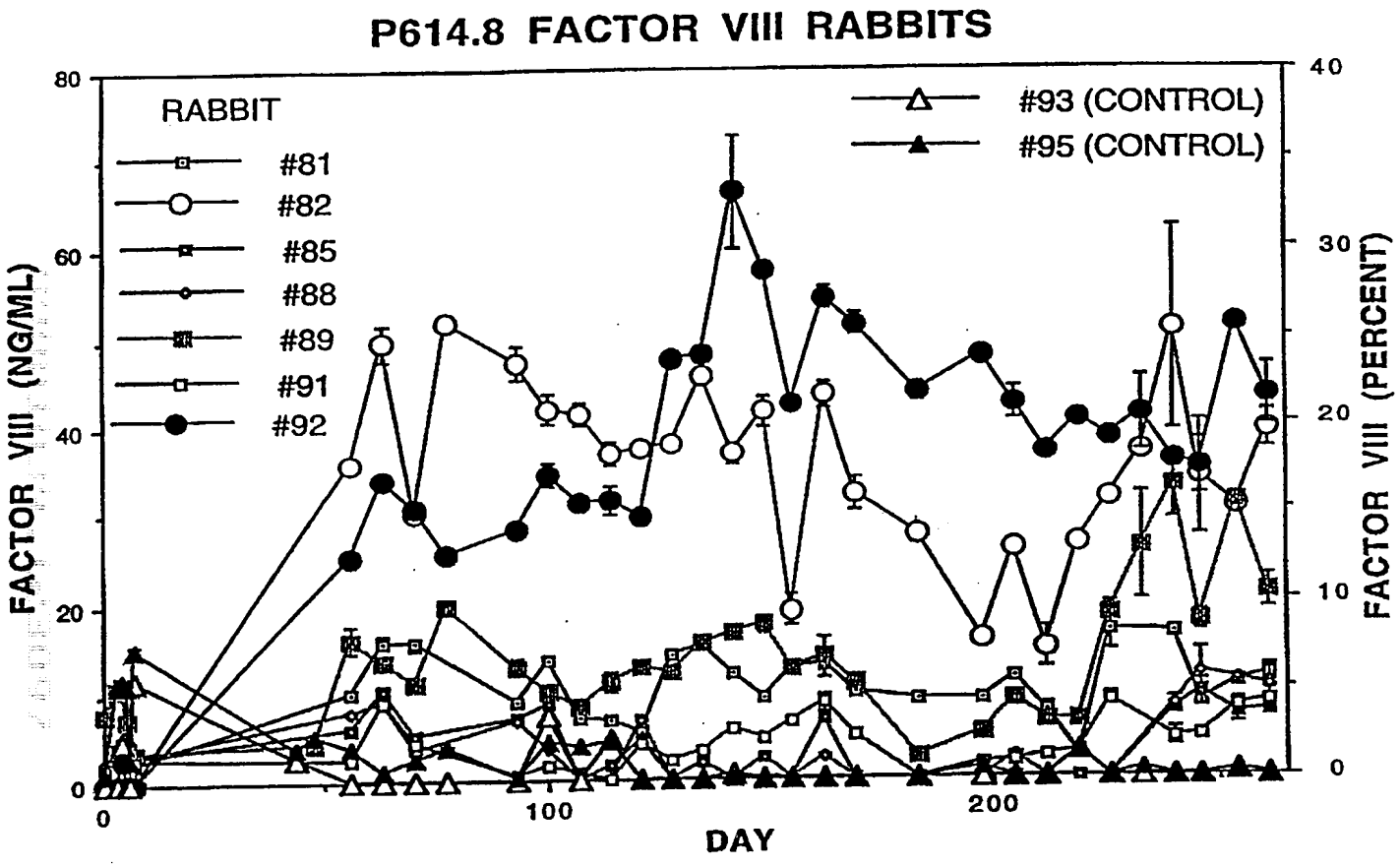

2e+8
2e+8
1e+8
5e+7
0e+0
BLUE CELL TITER
3
4
5
6
7
11
12
13
14
14
CULTURE DAY
HIGH SEED 72 hr TITER
HIGH SEED 48 hr EST. TITER
LOW SEED 48 hr TITER

FIG. 21

Fig 22


P614.8 FACTOR VIII RABBITS
RABBIT
#81
#82
#85
#88
#89
#91
#92
#93 (CONTROL)
#95 (CONTROL)
FACTOR VIII (NG/ML)
FACTOR VIII (PERCENT)
DAY
0
20
40
60
80
0
10
20
30
40
0
100
200

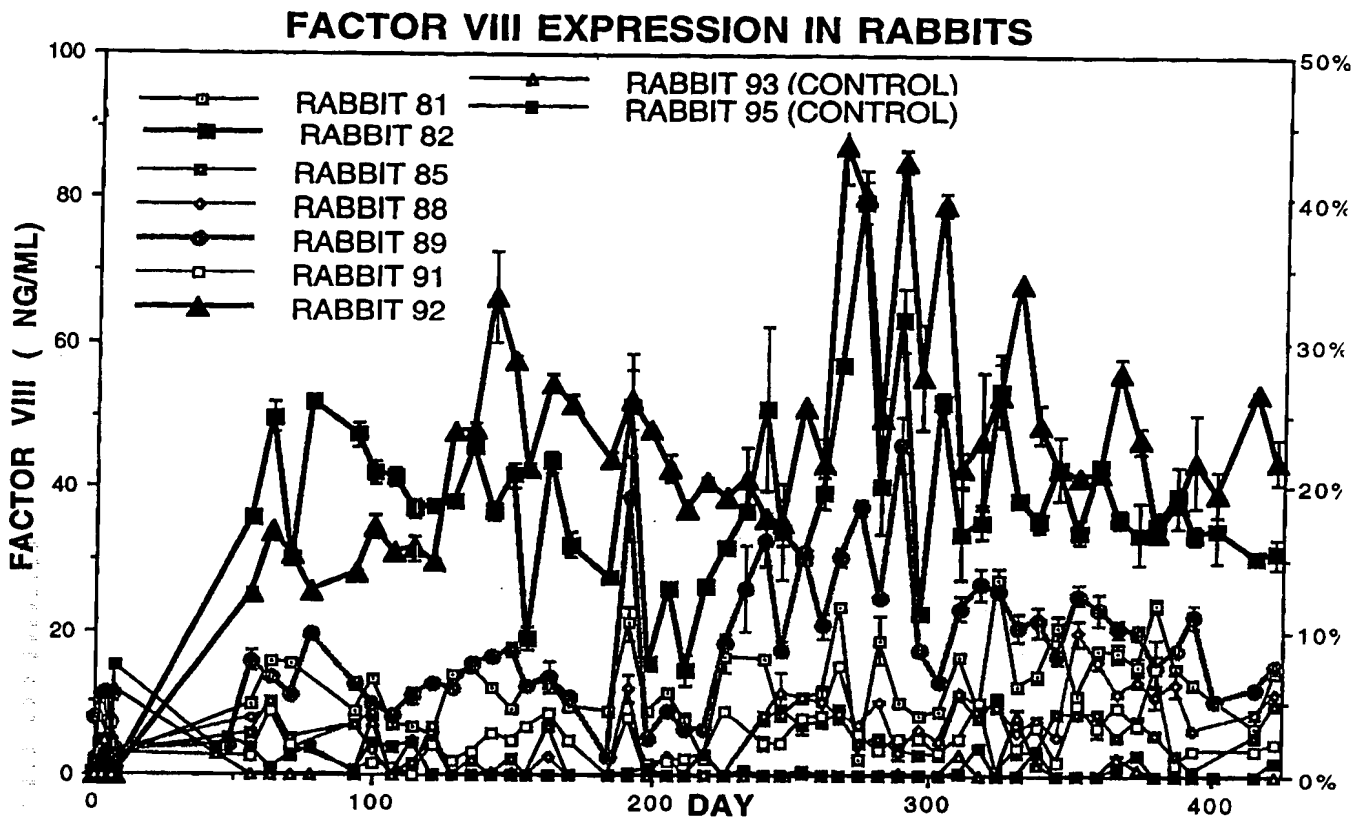

FACTOR VIII EXPRESSION IN RABBITS
FACTOR VIII (NG/ML)
100
80
60
40
20
0
50%
40%
30%
20%
10%
0%
0
100
200
300
400
DAY
RABBIT 81
RABBIT 82
RABBIT 85
RABBIT 88
RABBIT 89
RABBIT 91
RABBIT 92
RABBIT 93 (CONTROL)
RABBIT 95 (CONTROL)

Fig 23

SYSTEMIC HUMAN GROWTH HORMONE EXPRESSION IN RABBITS

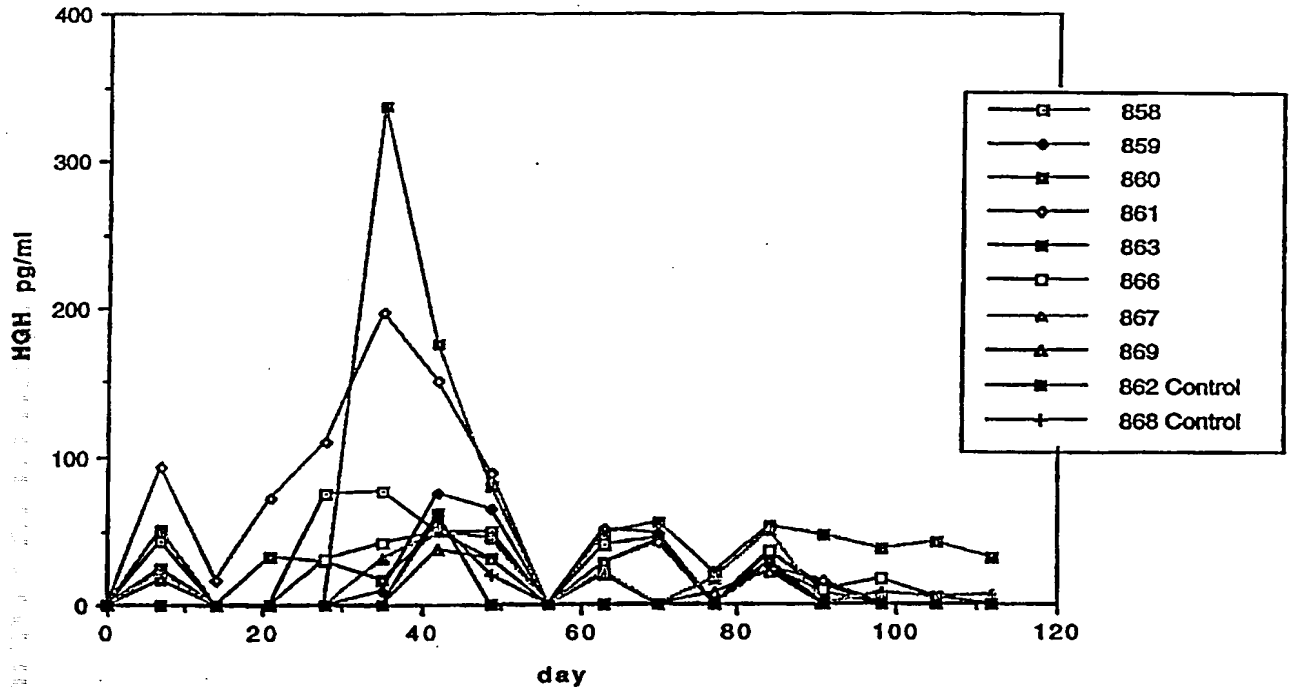
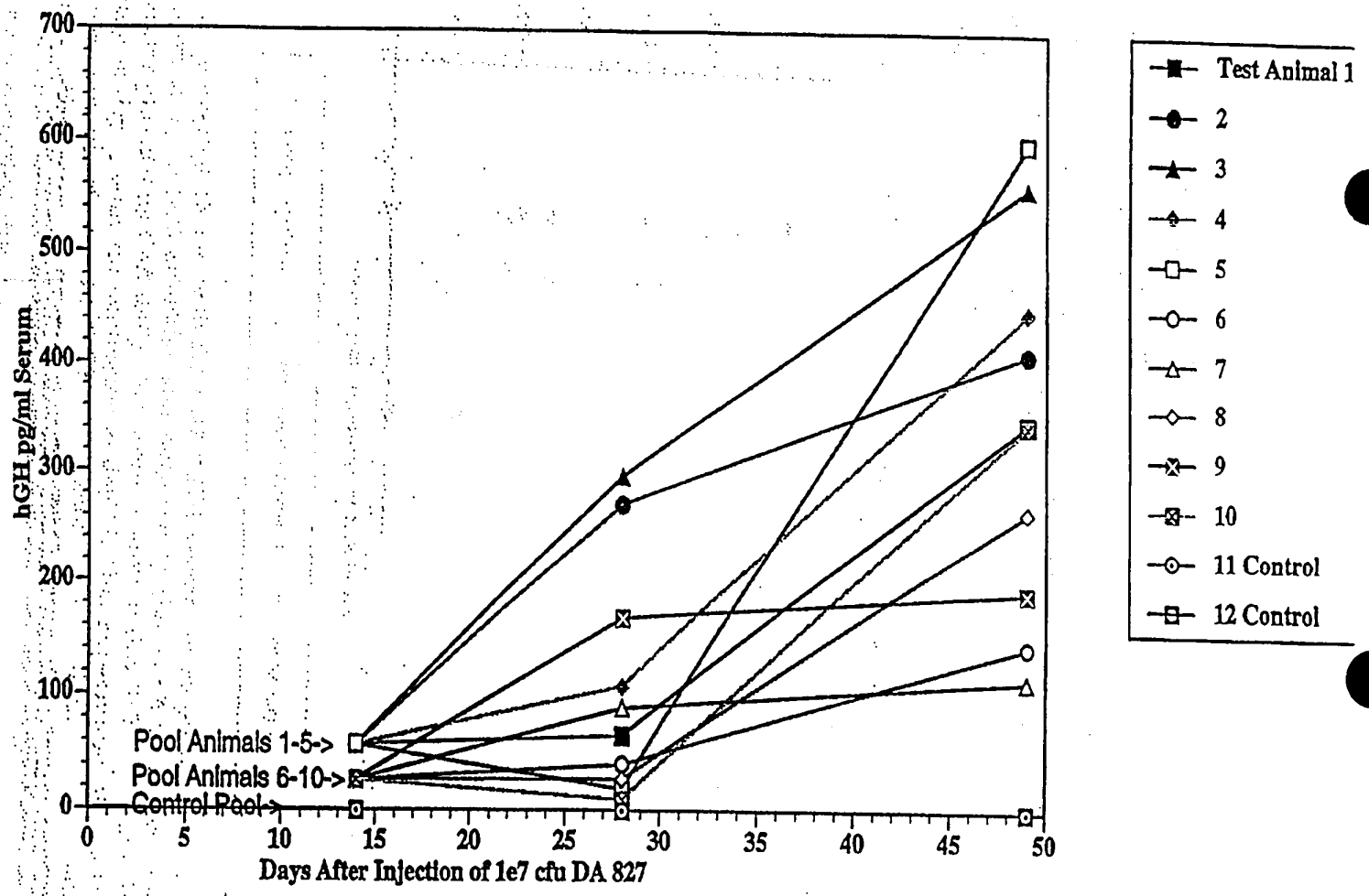


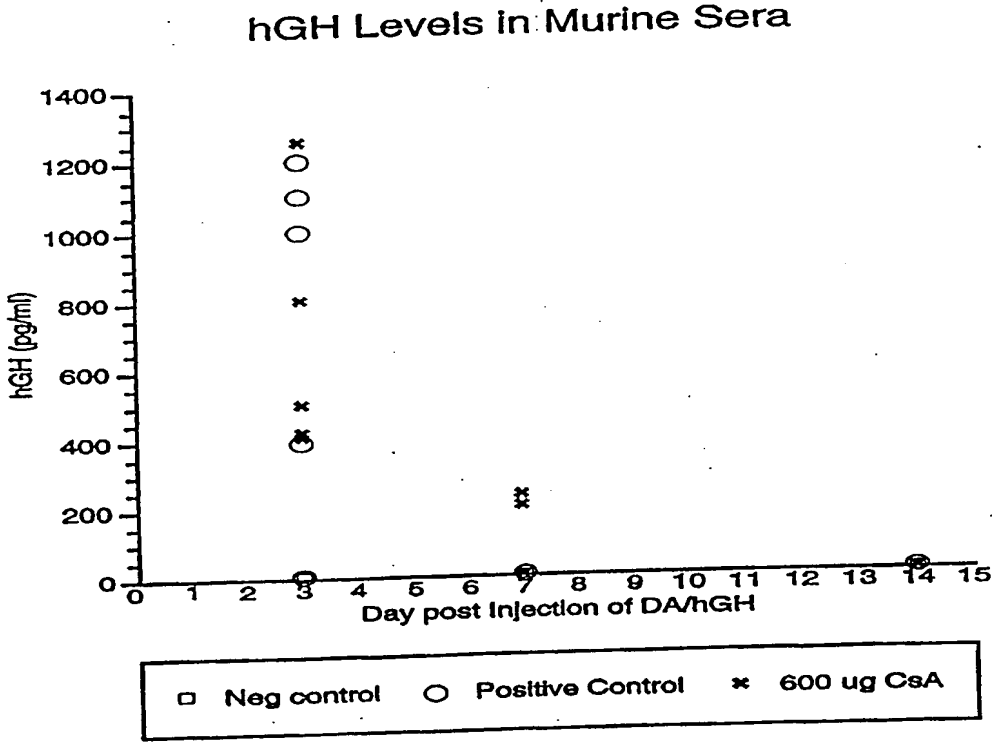
Fig 24

Time Course of hGH Expression in Mice Injected with DA 827



•Time Point 1 (Day 14) is an average of each pool.

Fig 26


hGH Levels in Murine Sera
hGH (pg/ml)
1400
1200
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Day post Injection of DA/hGH
Neg control
Positive Control
600 ug CsA

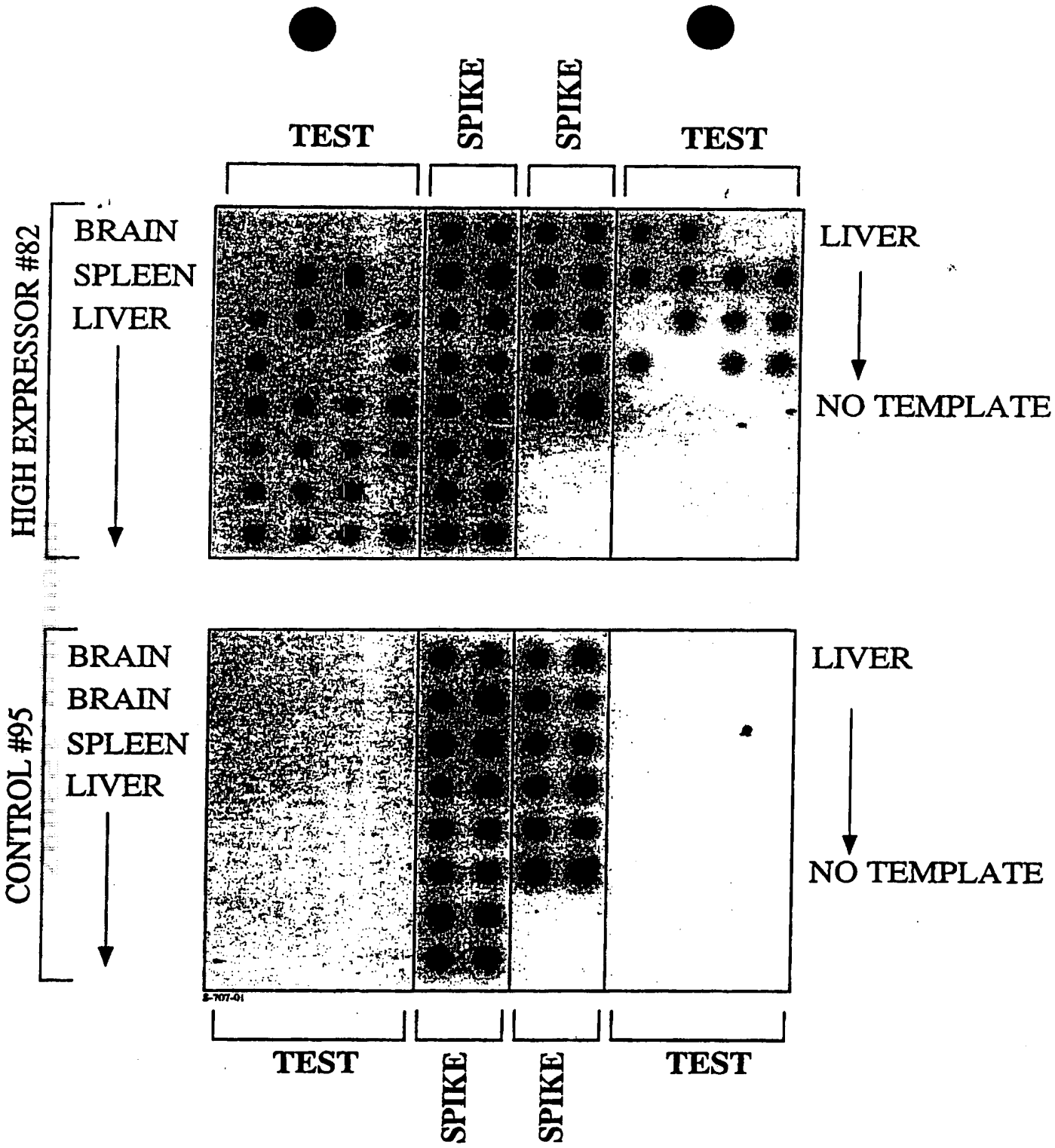

TEST
SPIKE
SPIKE
TEST
HIGH EXPRESSOR #82
BRAIN
SPLEEN
LIVER
LIVER
NO TEMPLATE
CONTROL #95
BRAIN
BRAIN
SPLEEN
LIVER
LIVER
NO TEMPLATE
TEST
SPIKE
SPIKE
TEST

Fig 27

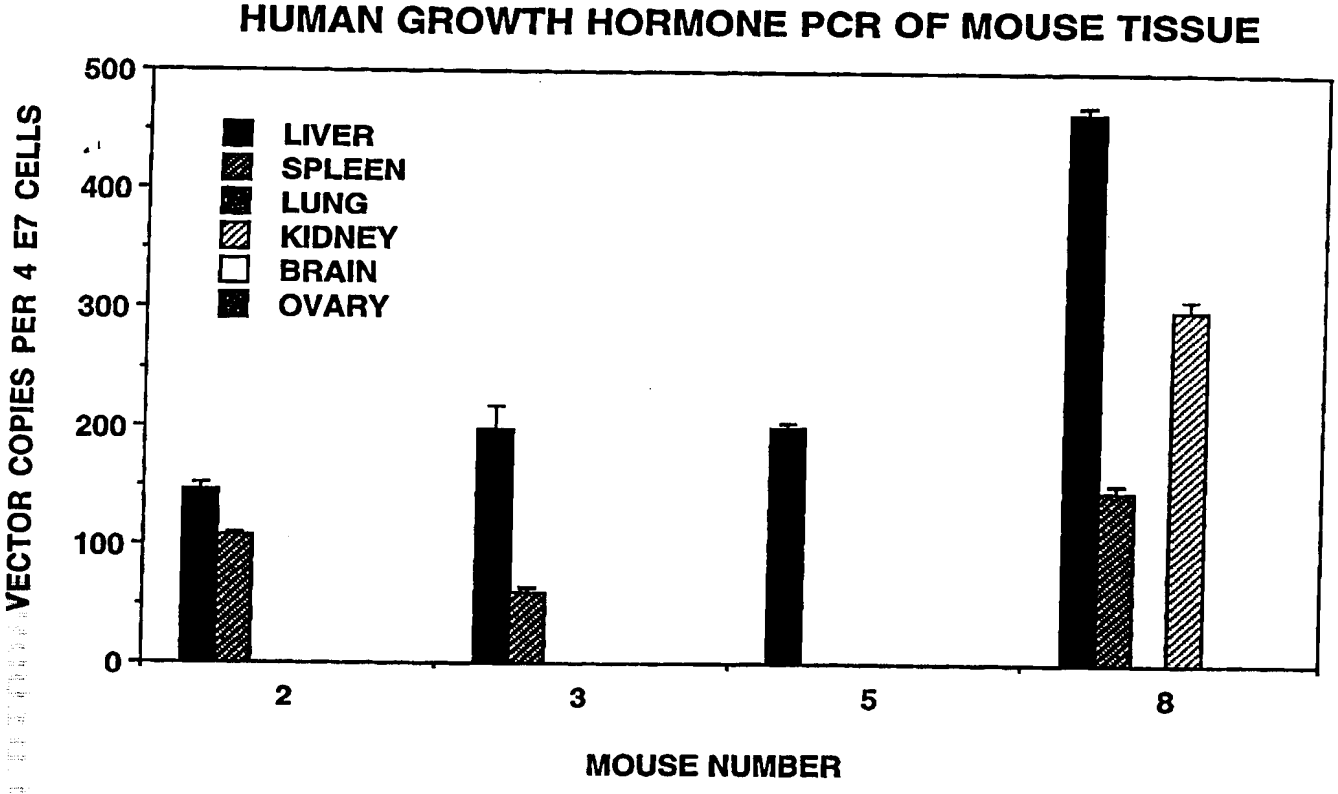

HUMAN GROWTH HORMONE PCR OF MOUSE TISSUE
VECTOR COPIES PER 4 E7 CELLS
500
400
300
200
100
0
LIVER
SPLEEN
LUNG
KIDNEY
BRAIN
OVARY
2
3
5
8
MOUSE NUMBER

Fig 28

EXPRESSION OF HGH PROTEIN IN MOUSE ORGAN LYSATES

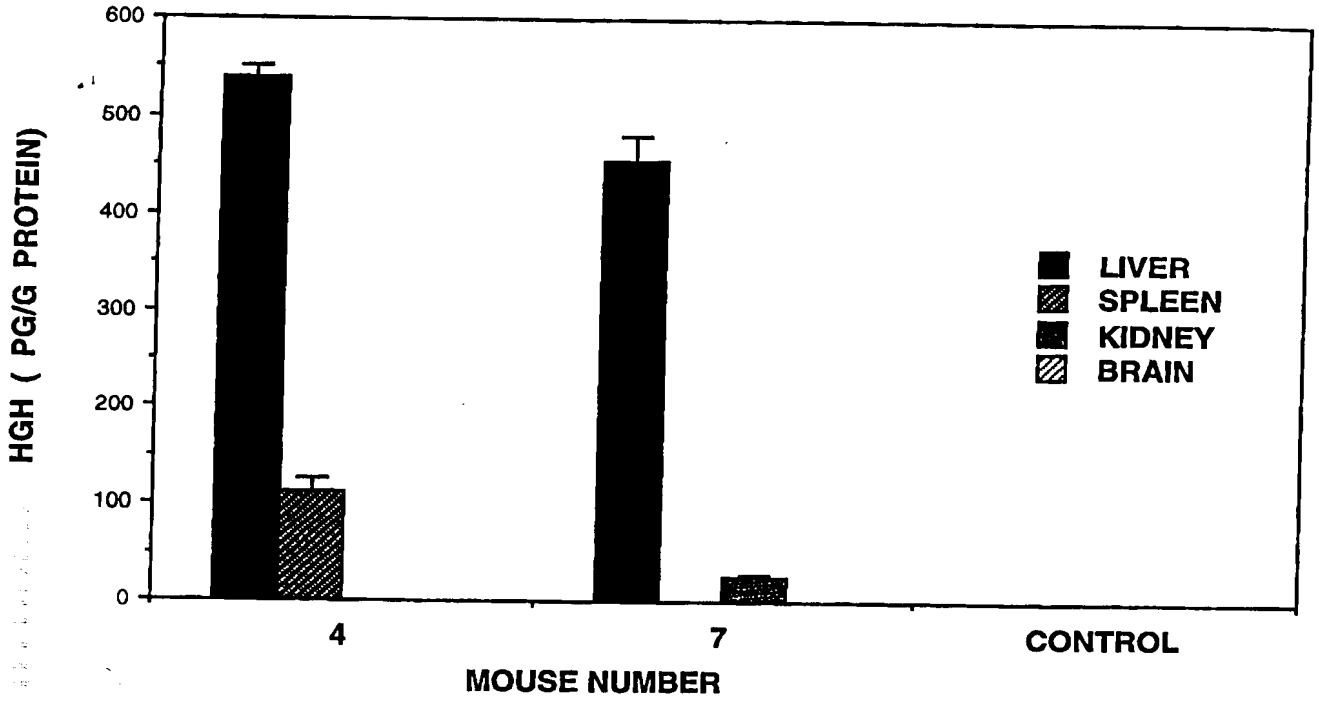


Fig 29

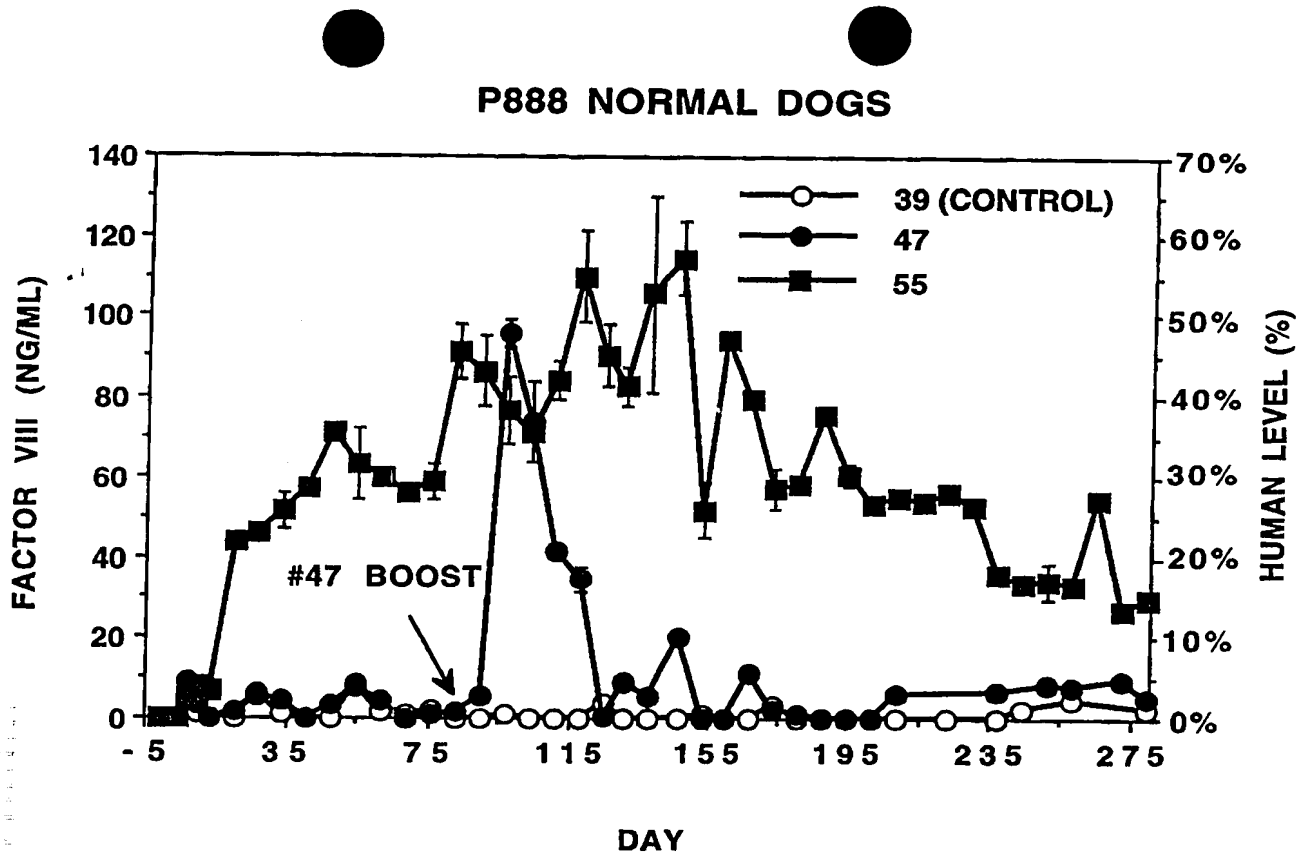

P888 NORMAL DOGS
FACTOR VIII (NG/ML)
HUMAN LEVEL (%)
DAY
39 (CONTROL)
47
55
#47 BOOST
140
120
100
80
60
40
20
0
70%
60%
50%
40%
30%
20%
10%
0%
- 5
35
75
115
155
195
235
275

Fig 30

Transfer of hGH Expression by Spleenocytes

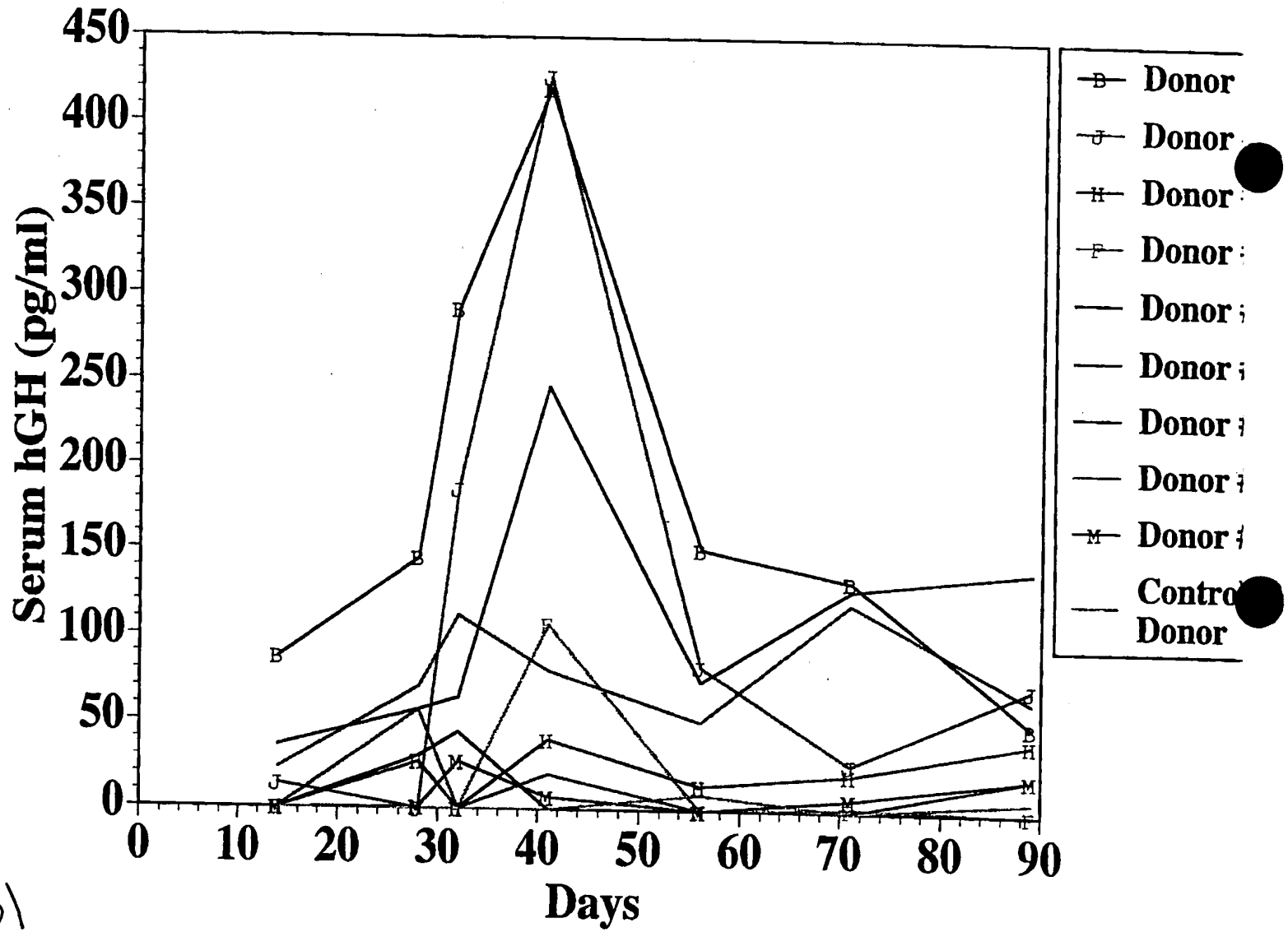


Fig 31

HEMOPHILIAC DOGS

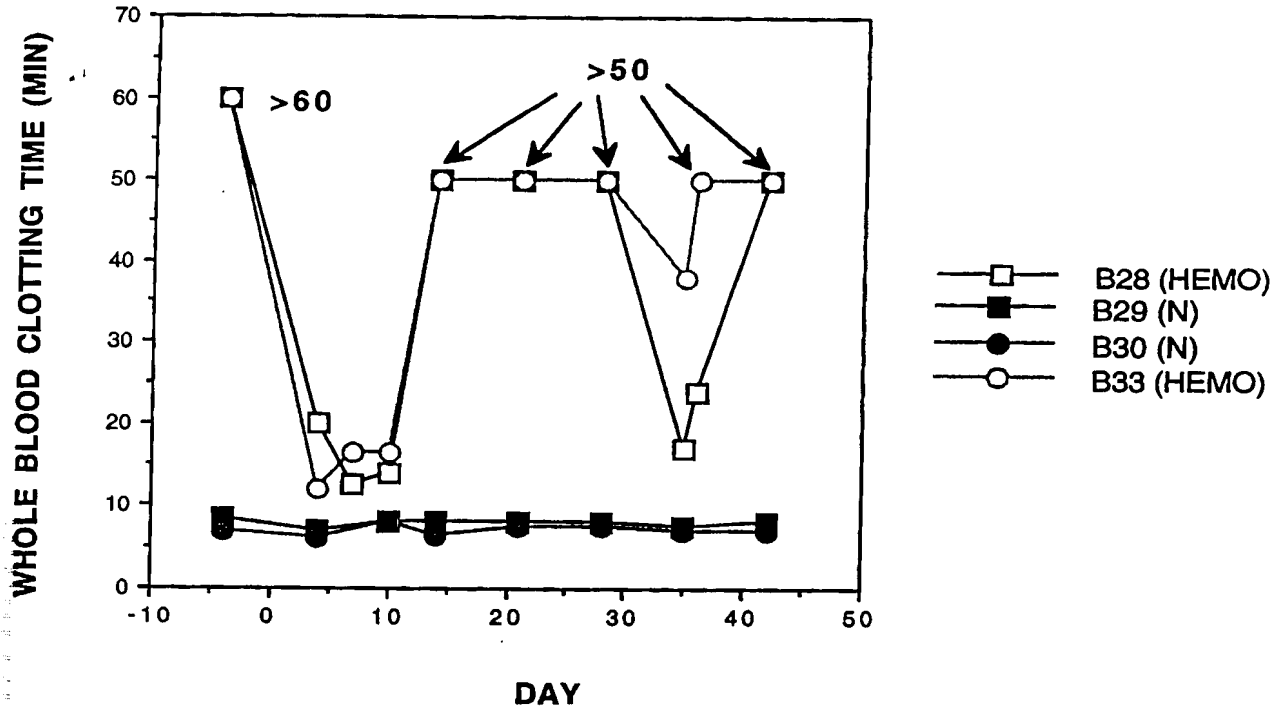


Fig 32

pKT1

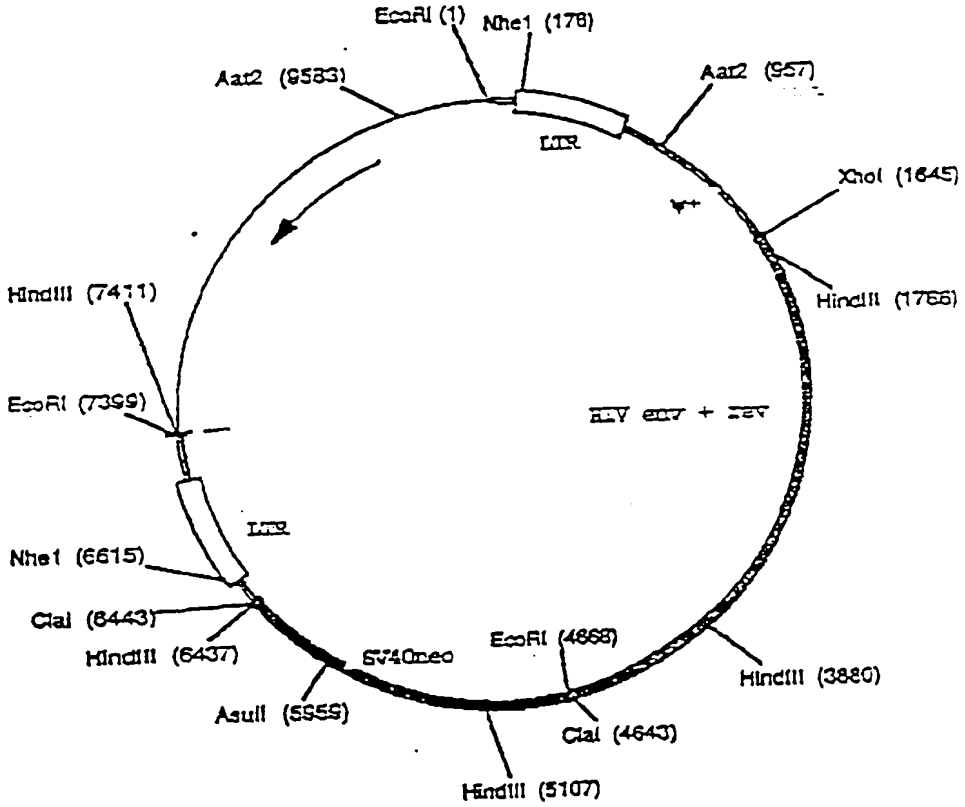
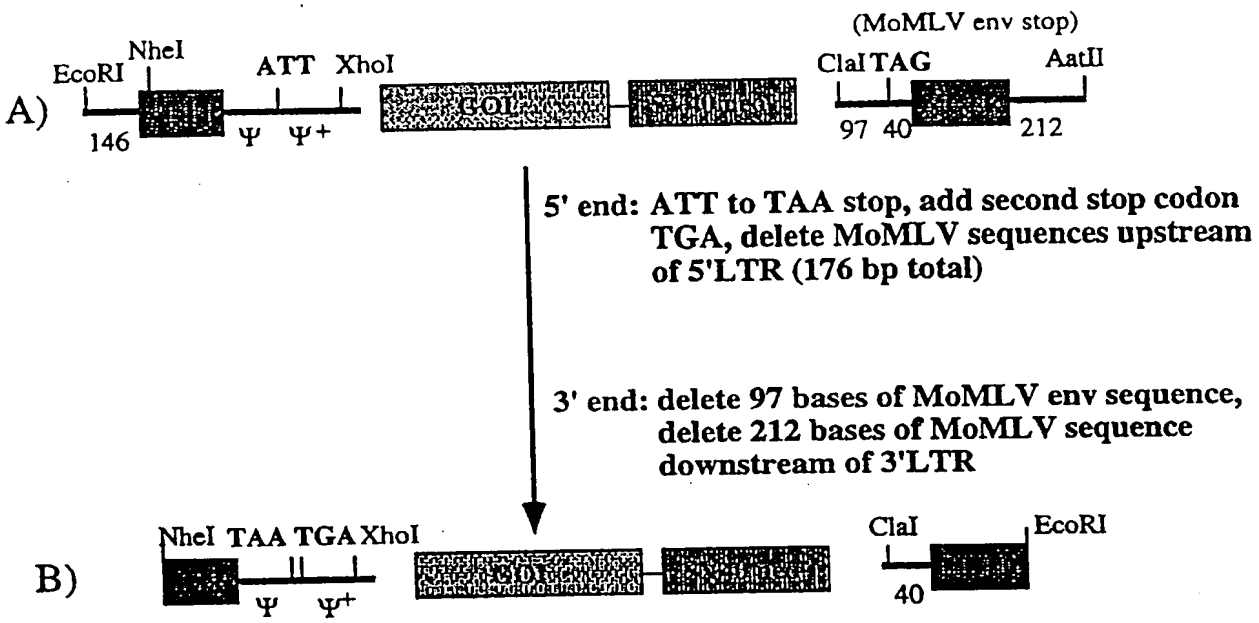

EcoRI (1)
Nhe1 (178)
Aat2 (9583)
Aat2 (957)
LTR
XhoI (1645)
HindIII (1766)
HindIII (7411)
EcoRI (7399)
LTR
Nhe1 (6615)
ClaI (6443)
HindIII (6437)
SV40neo
EcoRI (4668)
HindIII (3880)
AsuII (5555)
ClaI (4643)
HindIII (5107)

Fig 33

Fig 34

RETROVIRAL BACKBONE (N2-derived)



CROSS-LESS RETROVIRAL BACKBONE: pBA-5

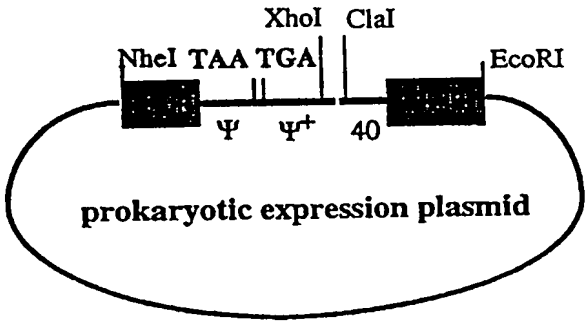


Fig 35

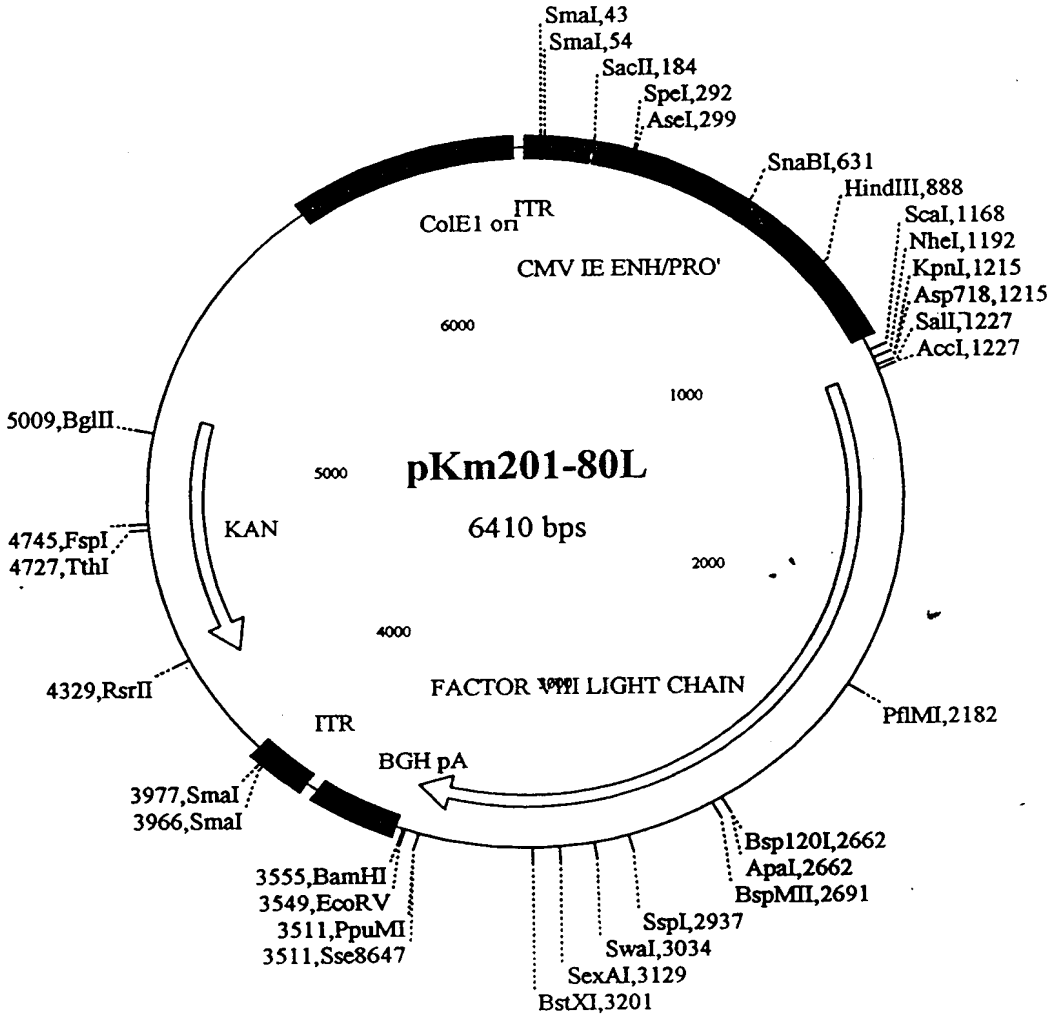

SmaI,43
SmaI,54
SacII,184
SpeI,292
AseI,299
SnaBI,631
HindIII,888
ScaI,1168
NheI,1192
KpnI,1215
Asp718,1215
SalI,1227
AccI,1227
ITR
ColE1 ori
CMV IE ENH/PRO'
6000
1000
5009,BglII
5000
pKm201-80L
6410 bps
KAN
4745,FspI
4727,TthI
2000
4000
4329,RsrII
FACTOR VIII LIGHT CHAIN
PflMI,2182
ITR
BGH pA
3977,SmaI
3966,SmaI
Bsp120I,2662
ApaI,2662
BspMII,2691
3555,BamHI
3549,EcoRV
3511,PpuMI
3511,Sse8647
SspI,2937
SwaI,3034
SexAI,3129
BstXI,3201

Fig 36

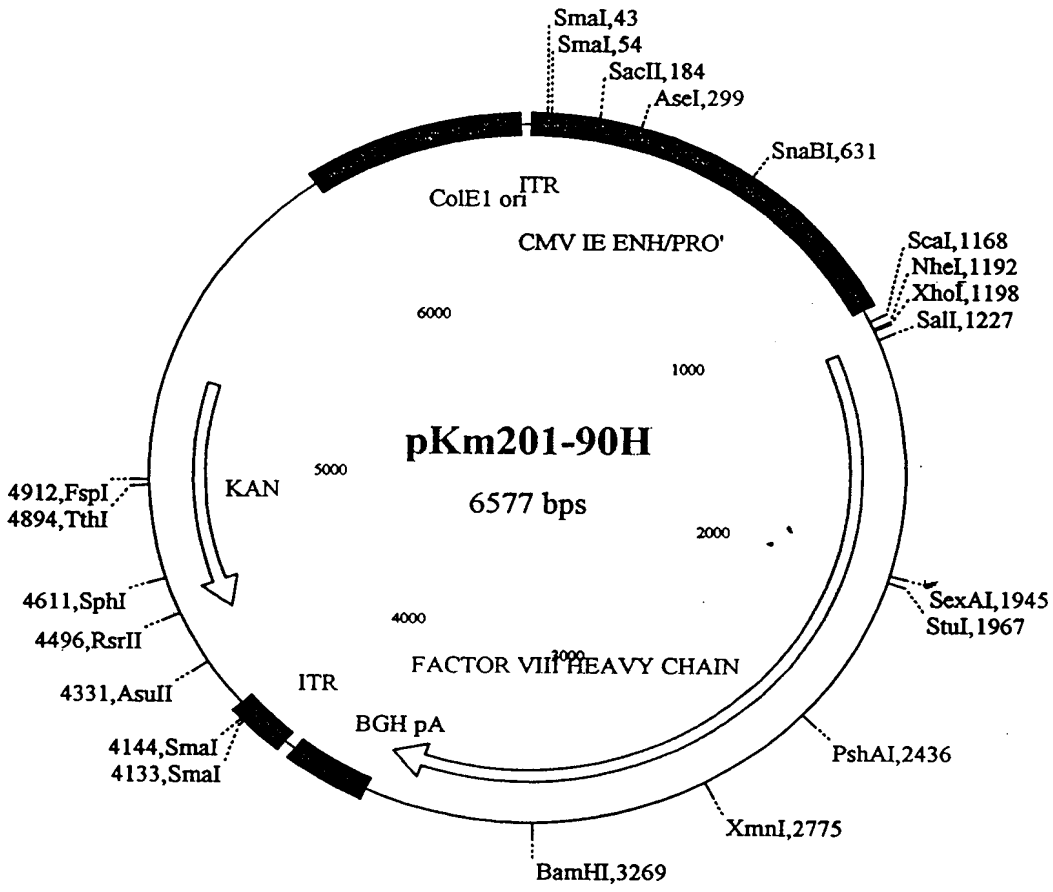
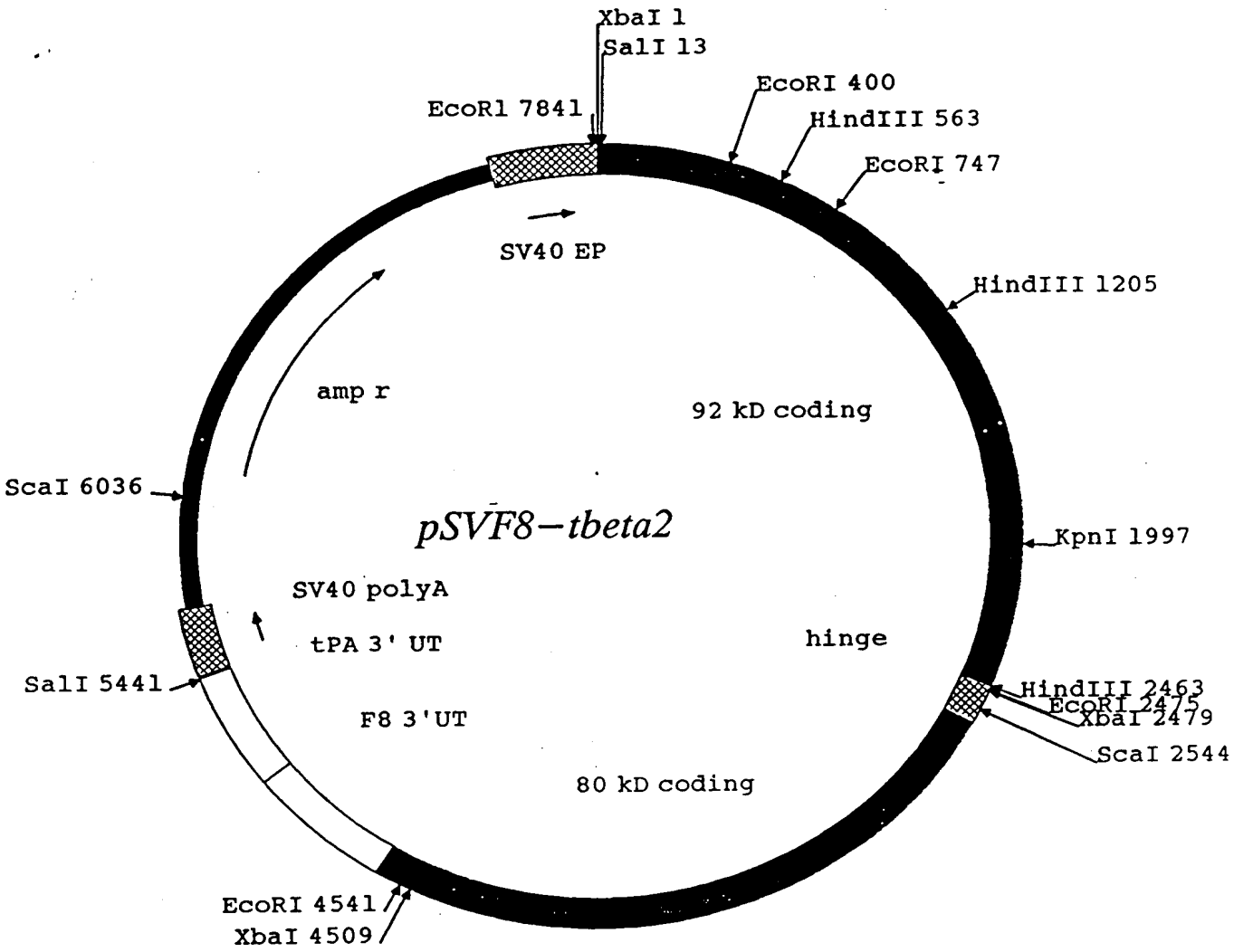

SmaI,43
SmaI,54
SacII,184
AseI,299
SnaBI,631
ITR
ColE1 ori
CMV IE ENH/PRO'
ScaI,1168
NheI,1192
XhoI,1198
SalI,1227
6000
1000
pKm201-90H
5000
KAN
6577 bps
2000
4912,FspI
4894,TthI
4611,SphI
4496,RsrII
4000
3000
FACTOR VIII HEAVY CHAIN
SexAI,1945
StuI,1967
4331,AsuII
ITR
BGH pA
4144,SmaI
4133,SmaI
PshAI,2436
XmnI,2775
BamHI,3269

Fig 37


XbaI 1
SalI 13
EcoRI 400
EcoRI 7841
HindIII 563
EcoRI 747
SV40 EP
HindIII 1205
amp r
92 kD coding
ScaI 6036
pSVF8–tbeta2
KpnI 1997
SV40 polyA
tPA 3' UT
hinge
SalI 5441
HindIII 2463
EcoRI 2475
XbaI 2479
F8 3'UT
ScaI 2544
80 kD coding
EcoRI 4541
XbaI 4509

```
                ArgGlyMetThrAlaLeuLeuLysValSerSerCysAspLysAsnThrGlyAspTyrTyr  Seq ID No. 48
2341            AGAGGCATGACCGCCTTACTGAAGGTTTCTAGTTGTGACAAGAACACTGGTGATTATTAC  Seq ID No. 49
                TCTCCGTACTGGCGGAATGACTTCCAAAGATCAACACTGTTCTTGTGACCACTAATAATG

                GluAspSerTyrGluAspIleSerAlaTyrLeuLeuSerLysAsnAsnAlaIleGluPro
2401            GAGGACAGTTATGAAGATATTTCAGCATACTTGCTGAGTAAAAACAATGCCATTGAACCA
                CTCCTGTCAATACTTCTATAAAGTCGTATGAACGACTCATTTTTGTTACGGTAACTTGGT

                   <---------- N-terminus of beta domain ---------------->

                ArgSerPheSerGlnAsnSerArgHisProSerThrArgGlnLysGlnPheAsnAlaThr
2461            AGAAGCTTCTCCCAGAATTCTAGACACCCTAGCACTAGGCAAAAGCAATTTAATGCCACC
                TCTTCGAAGAGGGTCTTAAGATCTGTGGGATCGTGATCCGTTTTCGTTAAATTACGGTGG

                2463 HIND3, 2475 ECORI, 2479 XBAI,

                <-- IgA hinge --->(-- C-term. beta domain -->

                ProProThrProProThrProProValLeuLysArgHisGlnArgGluIleThrArgThr
2521            CCTCCTACACCACCAACCCCACCAGTACTGAAACGCCATCAACGGGAAATAACTCGTACT
                GGAGGATGTGGTGGTTGGGGTGGTCATGACTTTGCGGTAGTTGCCCTTTATTGAGCATGA

                                2544 SCAI,

                ThrLeuGlnSerAspGlnGluGluIleAspTyrAspAspThrIleSerValGluMetLys
2581            ACTCTTCAGTCTGATCAAGAGGAAATTGACTATGATGATACCATATCAGTTGAAATGAAG
                TGAGAAGTCAGACTAGTTCTCCTTTAACTGATACTACTATGGTATAGTCAACTTTACTTC

                      2592 BCLI,
```

Fig 38

Fig 39

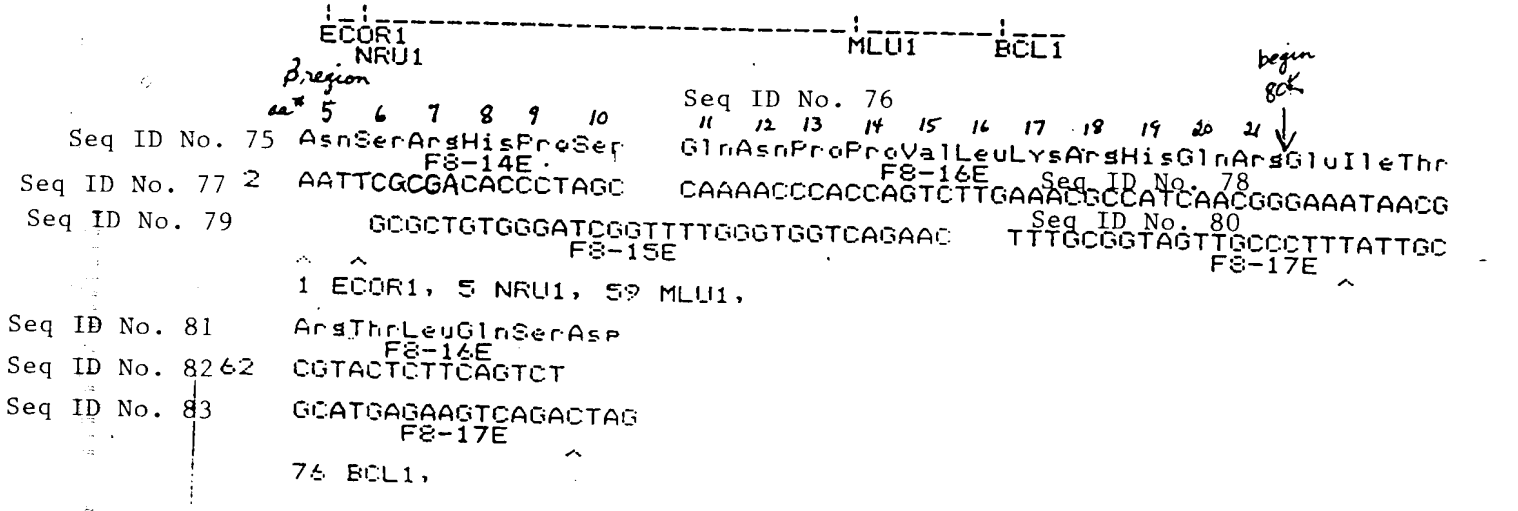
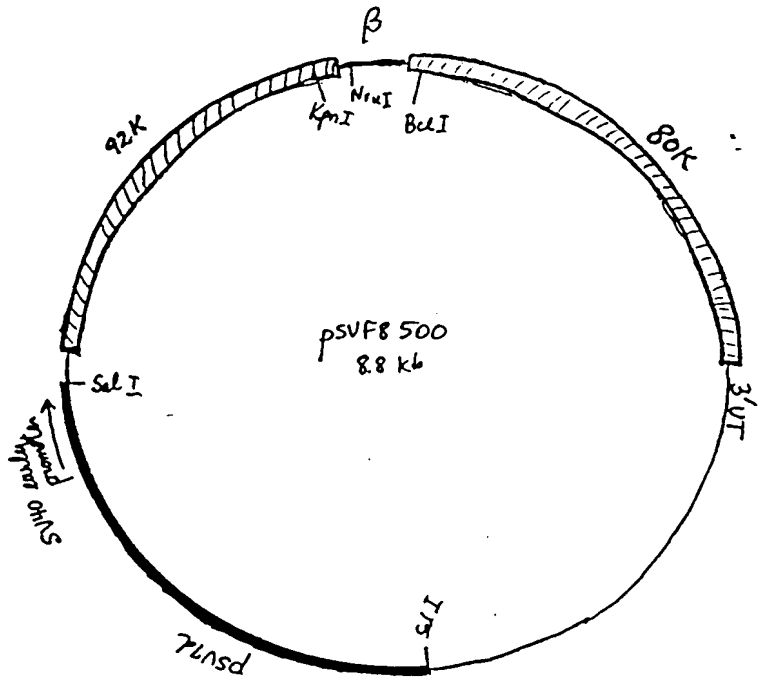
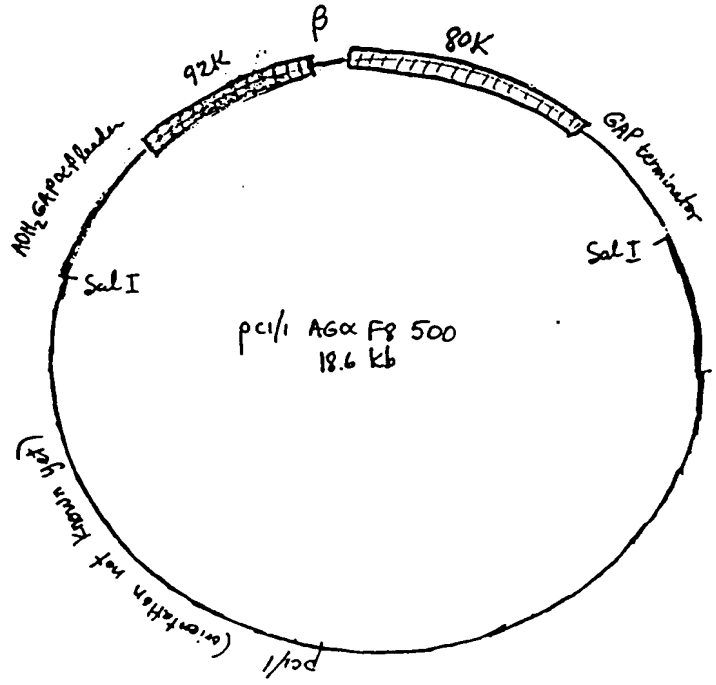

ECOR1
NRU1
MLU1
BCL1
β region
aa* 5 6 7 8 9 10
11 12 13 14 15 16 17 18 19 20 21
begin BCK
Seq ID No. 75 AsnSerArgHisProSer
F8-14E
Seq ID No. 76
GlnAsnProProValLeuLysArgHisGlnArgGluIleThr
F8-16E
Seq ID No. 77 2 AATTCGCGACACCCTAGC
CAAAACCCACCAGTCTTGAAACGCCATCAACGGGAAATAACG
Seq ID No. 78
Seq ID No. 79
GCGCTGTGGGATCGGTTTTGGGTGGTCAGAAC
Seq ID No. 80
TTTGCGGTAGTTGCCCTTTATTGC
F8-15E
F8-17E
1 ECOR1, 5 NRU1, 59 MLU1,
Seq ID No. 81 ArgThrLeuGlnSerAsp
F8-16E
Seq ID No. 82 62 CGTACTCTTCAGTCT
Seq ID No. 83 GCATGAGAAGTCAGACTAG
F8-17E
76 BCL1,

Fig 40


β
92K
KpnI
NruI
BclI
80K
pSVF8 500
8.8 kb
SalI
3'UT
SV40 promoter
pSV2d
SalI


β
92K
80K
ADH2 GAP promoter
GAP terminator
SalI
SalI
pc1/1 AGα F8 500
18.6 kb
pc1/1 (orientation not known yet)

Linkers for pSVF8-500B

end 92 19aa C terminal
to thrombin cleavage at 740

SerArgHisProSerThrArgGlnLysGlnPheAsnAlaThrProProValLeuLysArg Seq ID No. 50
TCGCGACACCCTAGCACTAGGCAAAAGCAATTTAATGCCACCCCACCAGTCCTGAAACGC Seq ID No. 51
mutant AGCGCTGTGGGATCGTGATCCGTTTTCGTTAAATTACGGTGGGGTGGTCATGACTTTGCG
wild type (TT) (CT)
NRU1

Start 80K
HisGlnArgGluIleThrArg
CATCAACGGGAAATAACGCGT
GTAGTTGCCCTTTATTGCGCA

MLU1
9aa N terminal to 80K

Fig 41